

Should Science Proceed Uncontrolled?

SCIENCE, as Peter Medawar put it, is the art of the soluble. Scientists do not venture forth into schemes of research without some indication that they will ultimately be successful in their work. This generally means two things: that their research must be completed in a reasonable amount of time by a reasonable number of people, and that it will not involve too many steps into the unknown at once. It is foolish to attempt to carry out jobs for which there is a serious lack of tools, experimental, mathematical or conceptual. Most research workers find it enough of a struggle to extend the capabilities of, say, electron microscopy without simultaneously looking for a digital computer that is a thousand times bigger than any existing at present and developing for themselves a mathematical framework within which to work. Research thus tends to be timely, not only in the sense of answering present needs, but in the sense of being appropriate to the competence of the times in which it is set.

This unromantic view of science—a view which nonetheless does not exclude or belittle the Newtons and Einsteins—raises an interesting question. Scientists in some sense regulate themselves by going for the soluble—the only way to remain sane and relatively satisfied. Science itself, however, has no comparable regulatory process to ensure that its growth is along the right lines.

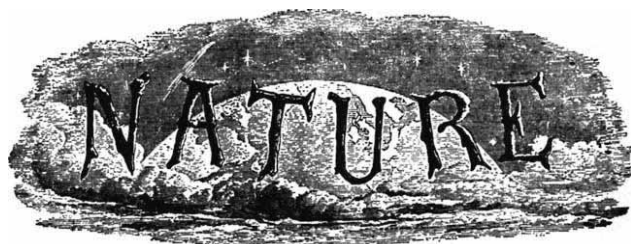
Many would argue, of course that talking about right lines at all is a nonsense. Science, they would say, is what scientists care to make it by their research, and it is nobody's business to organise other people's labours in order to cause science to develop along the lines of some personally conceived grand scheme. Certainly one cannot but have sympathy with those subjected to Five Year Plans, Global Strategies, Milestones and other orderly ideas imposed on some branches of science that have been blessed with tidy-minded bureaucracy at the top. Such rigidity often seems to discount creativity in science and to equate a healthy science solely with one in which there are enough data to go round. Nevertheless the excesses of regulatory zeal manifested in certain places should not detract from the need to ask the question whether the development of science should be entirely freed of controls. Every year universities turn out hundreds of PhDs, possessing, in theory, the skills and qualifications to do scientific research. If their work comes within the sphere of influence of the customer/contractor relationship they will experience certain forces which bear some resemblance to market-place economics and they cannot afford to be too cavalier in their disregard of the customer even though he may not have a very clear idea of what he wants, only of what he can afford. If, on the other hand, they can stay in some university department into which the customer has not yet ventured they can be free of many external forces that might seek to dictate the content of their research. Anything that is soluble is permissible.

It may be objected that the peer review process prevents excesses from happening. Research work comes under

formal scrutiny by grant-giving bodies before it is started and again by the referees of learned journals when it is completed. It comes under more informal review in the continuous circulation of scientists around the community. Is this not adequate control? The difficulty with the peer review process is that it only affords the reviewer a very limited view of the science, as opposed to the scientist, and ultimately his answer must be yes or no. He may wish to say that the applicant be encouraged to work on a different problem. He may wish to have the perspective of seeing who else is applying, in order to ensure that money is most effectively spent. He may even, if sufficiently energetic, wonder about how the proposed work compares with proposals being made in other countries, since science supposedly transcends national frontiers. But how often is it possible to do anything about these broader issues? Likewise referees are frequently faced with manuscripts which are impeccable but which, if published, will be neither read nor used. The dilemma seems always to be that of being thought arrogant if one speaks one's mind and stands up for a particular view of the development of science. Yet, privately, scientists can regularly be heard to bemoan the way in which bandwagons are followed to the detriment of less spectacular, but very necessary, research, students are encouraged to move into already overcrowded fields and so on.

It is asking a lot of administrations for them to define guidelines for scientific as opposed to administrative direction. It is better that practising scientists—unfortunately the very ones who can least spare time from their research activities—should be deeply involved. Money for scientific research is not going to flow ever more freely in the future and scientists must ensure that their opinions are heard now about the way in which science should develop rather than risk having administrative and political solutions imposed on them from above.

100 Years Ago



MR. JAMES DALLAS, of Benakandy, Cachar, writing us on the subject of inherited peculiarities, says that a friend of his has a black-and-tan English terrier dog, two inches of the end of whose tail is folded back so acutely as to come in contact with the upper portion. A pup, of which the dog is the undoubted father, has inherited the paternal peculiarity, with the difference that, instead of the end of the tail being turned up, it is turned down.

From *Nature*, 9, 17, November 6, 1873.



NOBEL PRIZES

Physics Award for Tunnelling Research



Dr. Brian Josephson, a particularly young winner of the Nobel Prize.

THIS year's award of the Nobel Prize for Physics to Leo Esaki, Ivor Giaever and Brian Josephson recognises the importance of tunnelling phenomena in semiconductors and superconductors. According to the quantum theory, electrons have a small probability of tunnelling through a potential barrier which is impenetrable according to classical theory. The phenomena depend on this probability.

Semi-conductors or super-conductors

The tunnel diode, discovered by Esaki (University of Tokyo) in 1957, is a p-n junction in a semiconductor so heavily doped that both sides are degenerate. The space-charge layer in the junction forms the potential barrier. When a small voltage is applied to bias the junction in the forward direction electrons tunnel from the n side to the valence band on the p side. When the bias voltage is sufficiently great, however, the electrons on the n side will have energies within the energy gap on the p side; since there will be no allowed states to receive the electrons, the current will drop and the diode will have a negative resistance. When the forward bias is sufficiently large the electrons will be able to tunnel across to the conduction band on the p side, and the resistance will become positive again. These properties of the diode enable it to be used in high speed amplifiers and oscillators. Since the electron current is affected by other excitations in the semiconductor, the diode can be also used to study these

excitations.

Giaever, originally Norwegian and a mechanical engineer, switched to physics on joining the General Electric Research Laboratory, New York. Impressed by the analogy with semiconductors, he used tunnelling to study the superconducting energy gap. In this case, the potential barrier was an insulating layer (usually an oxide) sandwiched between two metals. If one metal is normal and one superconducting (the NIS sandwich) and if the temperature is low, electrons will be able to tunnel from normal metal to superconductor only if the voltage bias is sufficient to carry them over the gap. In 1960 Giaever announced the first observation of the energy gap by this method. Giaever also realised that there is an analogy between the superconductor/insulator/superconductor sandwich and the tunnel diode and that the former should, under suitable conditions, also have a negative resistance characteristic. This he observed and the effect is now being developed in devices. A fuller account of Giaever's discovery has been given by Schmitt in *Physics Today* (14, 38; 1961).

Subsequent theoretical work showed that the characteristic of the NIS sandwich provides a direct measure of the density of electronic states of a superconductor. Because of the effect of other excitations, notably the phonons, on the density of states the tunnelling characteristic can be used to study these other properties. This has helped to confirm the basic theory of superconductivity as well as to yield important information on the phonon spectra of superconductors.

Tunnelling Supercurrents

In 1962, in a two-page letter to *Physics Letters*, Josephson (University of Cambridge), then aged twenty-two, predicted that it would be possible for supercurrents to tunnel between superconductors and that this tunnelling would be accompanied by a number of interesting effects. Essentially, Josephson showed that the whole superconducting sandwich was a single superconductor governed by a single wave

function, and that there were low-lying many-electron states that carried small currents without dissipation. If a large current passed through the sandwich, a voltage V would appear across it and there would be a non-dissipative contribution to the electric current oscillating at the frequency $2eV/h$ independent of the material. If such a junction is placed in a microwave field, this supercurrent leads to steps in the current-voltage characteristic. Josephson also stated (and showed in detail in later papers) that a magnetic field would modulate the phase of the wave function across the junction in a way analogous to that in which the phase of a light wave depends on path length. The result is that the dependence of the maximum Josephson supercurrent through a junction on magnetic field produces a 'Fresnel diffraction pattern'. The story of Josephson's discovery has been told by Anderson in *Physics Today* (23, 23; 1970).

At the time John Bardeen was sceptical that the effect would be as large as Josephson predicted. At an international conference in 1962 there occurred one of those historic moments in physics, the young Josephson defending his theory against the doubts of the 'elder statesman'. Within a few months, experiment had vindicated Josephson.

Since that time many beautiful experiments have been performed that demonstrate the effects of a magnetic field on Josephson tunnelling and which show that the phase of a superconducting wave function is coherent over macroscopic distances (at least of the order of centimetres). The effect is the basis of sensitive devices for measuring magnetic fields, voltages and small currents, and junctions are under development as computer elements.

The a.c. Josephson effect has been detected and used to provide the most accurate value of the fundamental constant e/h ; this, in turn, has led to a reassessment of the values of the other fundamental constants. It is also used to provide a new voltage standard and is being developed for the generation, detection and mixing of electromagnetic waves.

Professor G. Rickayzen of the University of Kent at Canterbury describes the research work for which the Nobel Prize for Physics was awarded.

Recognition for Organometallic Chemistry

Professor P. L. Pauson of the University of Strathclyde summarises the work on organo-transition metal chemistry which has earned its two chief founders the 1973 Nobel Prize for Chemistry.

ERNST OTTO FISCHER and Geoffrey Wilkinson share this year's Nobel Prize for Chemistry for spearheading the spectacular advances in organo-transition metal chemistry during the past twenty years. Not only were they among the first to recognise in 1952 the unique structure of ferrocene but their success in synthesising other "metallocenes" and many related cyclopentadienyl-metal complexes opened up the whole field.

Sandwich Compounds

Wilkinson used the name "sandwich compounds" for the metallocenes to describe molecular architecture, a structure consisting of two parallel five-membered carbon rings (cyclopentadienyl groups) symmetrically arranged around the central metal atom. The rings may be regarded as having sextets of π electrons and the important consequence, the aromaticity of ferrocene, was recognised and demonstrated by R. B. Woodward. The extensive organic chemistry thus opened up continues to be widely explored, most intensively and successfully in A. N. Nesmeyanov's Moscow laboratory, whose recent work has revived interest in ferrocene derivatives as anti-anaemic agents. Despite some early commercial promise, for example, for vapour plating and as fuel additives—including anti-knock agents, where a cyclopentadienylmanganese compound has earned limited practical success—no major direct application has yet repaid the intense effort put into this field. Initially industrial interest was so strong that it was not uncommon for a new type of cyclopentadienyl complex to be synthesised independently in three industrial laboratories as well as in Fischer's and Wilkinson's laboratories. But the work of Fischer and Wilkinson gradually diverged as the field broadened.

Theoretical interest in these remarkable structures was no less intense than practical interest, and gradually deepening understanding helped greatly in extending the whole area of research. J. Chatt and L. A. Duncanson had taken an important step in 1953 by applying

M. J. S. Dewar's π -bonding ideas to explain the structure of the organo-metallic compound known longer than any other—Zeise's ethylene-platinum salt. The relationship to this and the cyclopentadienyl sandwiches of other known compounds, including some acetylene complexes first prepared by W. Reppe and his group as well as H. Reihlen's butadiene-iron complex, emerged gradually. During this period both Fischer and Wilkinson worked on a variety of related alkene, diene and triene complexes.



Professor Geoffrey Wilkinson

Suspicion that the most puzzling of the earlier discoveries, F. Hein's "polyphenylchromium" compounds, might also belong in the "sandwich" class received striking confirmation when E. O. Fischer and W. Hafner succeeded in synthesising the prototype: bisbenzene-chromium. With the impressive range of other arene-metal complexes subsequently synthesised in his laboratory, this will undoubtedly continue to be regarded as Fischer's most distinctive and important contribution.

More recently, most of his work has been concentrated on the intriguing carbene complexes, first prepared in his

laboratory as a result of a most skilful and purposeful search for suitable synthetic methods. These studies have taken him back much closer to the metal carbonyls, on which he worked as a student under W. Hieber, whom he succeeded in the chair of inorganic chemistry at the Technische Hochschule of Munich in 1964.

Fischer's entire scientific work to date has been done there and at the University of Munich (1957–64). He resisted calls to other chairs which would have taken him from his native city and further from the Alps which he loves and where he relaxes—usually in the company of his students, walking and skiing from an attractive chalet built with the proceeds of the bis-arene-chromium patents.

Return to Imperial College

Wilkinson—a Yorkshireman born in Todmorden—likewise holds office in his own alma mater, the Imperial College of Science and Technology, London. He returned there in 1956 after thirteen years in North America, having gone to Canada during the war to do radio-chemical work in connection with the development of the atomic bomb and moving by way of California to the Massachusetts Institute of Technology and then Harvard.

Most of his "sandwich" work was carried out at Harvard where Al Cotton, co-author of his famous textbook, was the first student to work with him in this field. Among Wilkinson's contributions of that period, his use of the then quite new NMR techniques demonstrated their great potential in this field and led to later discoveries of many interesting aspects of the behaviour of organometallic molecules. Since returning to London, his interests have gradually moved to other areas, notably the transition metal alkyls (he has devised molecules of almost as surprising stability as the original "sandwiches") and catalysis (he found and studied some of the most active homogeneous hydrogenation catalysts).

It is in such catalytic aspects, and in the use as chemical intermediates of such metal complexes as Fischer's carbene derivatives, that greatest interest and promise of use resides at present; but regardless of future developments, this year's Nobel Laureates have added a remarkable chapter to chemical knowledge.

Short Notes

Mark V—A Sound Investment

DETAILS and engineering drawings for the Mark V "Jodrell Bank" radio telescope have now been sent out for tender. Tenders will be in early in January, 1974, said Professor Sir Bernard Lovell last week, and a decision on whether to go ahead with building the telescope will be made by the SRC next spring. Sir Bernard also said that the staff at Jodrell Bank are "very happy" with the final design, and he is confident that the high accuracy required for its construction can be achieved. Although the final decision on building the instrument cannot be made until tenders have been considered, there is an air of optimism at Jodrell Bank, engendered partly by the recent granting of formal planning permission to build the telescope in the Meifod valley.

Outside Manchester, there have been reports that this optimism might yet prove unfounded, since the SRC is at present considering two expensive astronomy projects, the Mark V and the Northern Hemisphere Optical Observatory (NHO). Opponents of the Mark V argue that if the SRC is forced to choose between these projects then the radio telescope is more likely to be axed, because of the rising cost of steel, many thousands of tons of which are needed in the construction of such an instrument. But that argument is a two-edged weapon; the Mark I telescope at Jodrell Bank is today worth more as scrap than it cost to build, and from that point of view, the Mark V is a more sound investment than the NHO. According to a spokesman for the SRC last week, however, both optical and radio astronomers can rest assured in the knowledge that "planning is proceeding on both these projects and there is no reason to suppose that a choice will have to be made".

BA Launches BASS

THE inaugural conference of the British Association Students' Section (BASS) next January is to be entitled 'The Social Responsibility of the Scientist within the Community' which is a fair summary of BASS's preoccupations, as it now sees them. Speakers will include not only Dr Magnus Pyke, secretary of the British Association, and Sir Peter Medawar but also Mr James Moorhouse, of Rio Tinto Zinc, who will put the industrialist's point of view.

The new section will be distinct from the British Association of Young Scientists (BAYS) and membership is not restricted to scientists or to members of universities. It will be chiefly concerned with the consequences of science rather than with acquainting the public with developments in science, which has

been one of the main objectives of the British Association itself.

The BASS plans to hold a yearly conference and to carry on year-round activities in local branches based on places of higher education. These will not attempt to compete with already established scientific societies in providing general lectures except in certain cases, such as the University of Exeter, where the scientific societies are felt to be inadequate; but they will be involved in projects decided at the previous conference. The projects may be on similar lines to those organised by the British Association proper—for example the study groups on the role of research in higher education, the ethics of biological research and so on. Certainly they will involve the collection and discovery of already available data rather than original scientific research, as there are no central funds available for branch activities. The BASS will provide for the first time a forum for student discussion of science-related topics at the national level; and its creation may mean a new lease of life for the British Association.

Drapes Off at the DoE

FOLLOWING the recommendations of the Select Committee on Science and Technology that government departments should give regular accounts of their research activities, the Department of the Environment has produced its first report on research and development (HMSO, £0.42). The report reveals that in 1973–74, a greater proportion of the department's research resources are to be devoted to planning and transportation, and to environmental pollution and resources, all at the expense of building and construction.

Research and Development in the Department of the Environment

	1972–73	1973–74 *
	(£ million)	
Planning and transportation	4.9	10.3
Building and construction	7.2	8.4
Environmental pollution and resources	2.5	5.8
Other expenditure †	2.6	2.7
Total	17.2	27.2

* Planned programme.

† Includes grants-in-aid to research associations, the Centre for Environmental Studies and so on.

The expenditure involved is shown in the table, which reflects the transfer of funds from the Natural Environment Research Council according to the Rothschild recipe and from the Department of Trade and Industry. Much of the department's research effort is centred on its four research establishments—the Building Research Establishment, the Transport and Road Research Laboratory, the Hydraulics Re-

search Station and the Water Pollution Research Laboratory, which between them employ more than 1,400 scientists and engineers.

Soyuz-Apollo Project

THE second round of preparatory talks for the joint Soyuz-Apollo project (scheduled for 1975) was held in Moscow from October 1 to 18, 1973, and dealt with problems of flight ballistics, coordination of the Soviet and American control centres and problems of crew transfer between the craft. In connection with the latter topic, the Soviet team revealed full details of the cause of the Soyuz 11 disaster of June 30, 1971.

Although as early as July 4, 1971 a *Pravda* article by Academician Boris N. Petrov (a leading figure in the Soviet space programme) hinted at mechanical failure, and although it was later announced that the death of the three cosmonauts was caused by loss of cabin pressure on re-entry, the details remained a secret. Now, apparently on the insistence of the United States team, it has been revealed that the firing of twelve explosive bolts to disconnect the re-entry capsule from the orbiting craft was more violent than anticipated, and accidentally loosened a safety valve cap, thus triggering an exhaust valve, resulting in total loss of cabin pressure within 45 seconds.

Ground tests after the accident revealed that it would take 27 seconds to close the valve by hand —17 seconds longer than the crew could have remained conscious. Earlier Russian reports that two of the cosmonauts were found "with hand raised" would suggest that such an attempt was made. Like the fire in which three Apollo astronauts died, the Soyuz tragedy seems to have been caused by a 'safety' mechanism that could not be operated with sufficient speed. Also, like the American disaster, it has led to modifications of the craft, which were tested out by a space-suited two-man crew in the 2-day Soyuz 12 mission of September 1973.

What is a Quark?

IN *Physics Reports* (8, 173; 1973) Lipkin quotes the following definition of a quark, from a standard German-English dictionary: Quark, m. curd, curds; slime, slush, filth; (fig.) trifle, rubbish, trash; (sl.) du verstehst einen—davan, you understand damn-all about it; den alten—aufzufrhen (fig.), stir up mud. -ig, adj. containing curds; dirty. -kase, m. soft or cream cheese.

This reminds one of the Prime Axiom of physics: everything is made up of smaller pieces. This, together with the Corollary (some of the pieces attract each other; some are repulsive), seems to sum up the subject adequately, although glossing over some details.

NEW WORLD

A Special Case for DDT Treatment?

by our Washington Correspondent

In the past few years, bewhiskered caterpillars of the Douglas fir tussock moth have been quietly munching their way through nearly a million acres of fir forests in the north-western United States. The moth suddenly began a population boom in 1971, defied all predictions that the outbreak would end this year from natural causes, and is about to present the Environmental Protection Agency (EPA) with a serious dilemma. Should the ban on DDT, which was imposed last year after a long and bitter debate, be temporarily lifted so that the caterpillars can be sprayed when they hatch next spring? Last week, while American troops were on alert around the world and a domestic crisis was gripping the United States, a sub-committee of the House Agriculture Committee took time to hear about the caterpillars.

The moth is always present in the Pacific North-West, but it is usually relatively untroublesome, existing in small numbers in balance with its natural predators. Every so often, however, for reasons that have never been adequately explained, the moth undergoes an explosive population growth. Usually the epidemic suddenly ends in the third year, when the caterpillars all die off from a virus infection which spreads through the eggs during the winter months, and these periods of heavy infestation are then followed by several years of relative quiet. This time the moth refused to follow its traditional three-year cycle. According to the Forest Service, at the last count the insect had defoliated to some degree 657,000 acres in Oregon and Washington State and another 125,000 acres in Idaho. The committee was told last week that as many as 1.5 million acres of forests could be stripped next year if the infestation is not checked. The foresters in the area and the lumber companies that make their living from the trees want to kill the insects when they hatch next spring with the only pesticide they know will do the job—DDT. This does not appeal to environmentalist organisations which fought long and hard to get DDT banned, or the EPA which is understandably reluctant to allow use of a pesticide which it banned only a year ago amid a blast of publicity and a barrage of complaints from farmers, pesticide manufacturers and some scientists. The choice is, however, not

simply a matter of DDT or no DDT, and it is greatly complicated by the moth's failure during the past year to conform to its previous population trends.

To appreciate the basis of the EPA's dilemma, a little knowledge of the life cycle of the moth is required. Tiny tussock moth caterpillars hatch over a period of about a month in late May and early June from eggs laid during late August and September. The caterpillars go through five to seven moults, increasing in size as they eat first all the new foliage growth and then old foliage; they pupate in August and emerge in 10 to 18 days as adult moths. The female moth is wingless and mating takes place on the cocoon; she lays some 200 eggs in a mass and dies within a few weeks. The eggs remain on the trees during the winter and hatch in the spring. Thus, if the caterpillars are to be attacked with a pesticide, the application must come just as they first emerge, before they have had chance to start munching the foliage, and the pesticide should ideally remain active for a month or so to kill caterpillars that hatch late.

When a population explosion takes place, it usually goes undetected during

the first year, but becomes painfully obvious when caterpillars start stripping the trees in the second year. If left alone, the outbreak tends to end abruptly in the spring of the third year, because of the explosive spread of a nuclear polyhedrosis virus which kills off some of the caterpillars at the end of their first moult (instar). The infection spreads because the dying larvae infect foliage which is eaten by other caterpillars during their second instar, and so on—it is calculated that if 10% of the caterpillars are infected during their first instar, the entire population will be virtually wiped out by the end of June.

The problem is, however, that although the virus infection kills off the outbreak abruptly, it does not complete its work until a fair proportion of the caterpillars have survived long enough to munch foliage, and trees are still stripped even in the third year of an outbreak. The usual practice has thus been to spray infested trees with DDT in the spring of the third year of an outbreak but that was stopped in 1969, when the Department of Agriculture (the EPA was not then formed) banned the use of DDT on forest land.

The present outbreak began in 1971, and, by rights, should have ended

BOLL WEEVIL

An End in Sight

by our Washington Correspondent

WHILE a verbal battle was taking place in Washington last week over the use of DDT (see accompanying story), the US Department of Agriculture announced that it has successfully completed a programme which proves that it is technically possible to eradicate the boll weevil in the United States. One of the most destructive insect pests in the cotton fields, the boll weevil is now attacked with more insecticide than any other pest, and its elimination could save cotton farmers up to \$200 million a year in lost production.

The Department of Agriculture's announcement was based on the results of a two-year study of the use of integrated pest management techniques in a 5,000 square mile area in Mississippi, Alabama and Louisiana. The experiment involved the application of pesticides, the use of sex pheromones and, finally, the release of large numbers of sterile male insects. First, several limited pesticide applications were made in late

autumn to kill the last generation of weevils to reproduce that year, and to reduce the population which was due to hibernate during the winter. Then, in the spring, male sex pheromones were used to bait traps in and around the cotton fields (the boll weevil is one of the few species of insects where the male, and not the female, produces sex attractants). Finally, sterile males were released in the cotton fields in numbers far greater than those of the males that had survived the onslaught of pesticides the previous autumn.

Surveys conducted at the end of the second year of the experiment indicated that there was no evidence of boll weevils in 235 of the 236 cotton fields in the core of the experimental area. It should be pointed out, however, that it will be several years before the weevil can be eradicated by these techniques, because a national elimination plan must be formulated and approved by all the parties involved. Nevertheless, the experiment has raised hopes that one of the most destructive pests in the United States may at last be on the way out.

abruptly with a massive infection in June this year. Nevertheless, in March, the United States Forest Service and the States of Washington and Oregon petitioned the EPA to allow the use of DDT on the outbreak this spring. They wanted to use 200,000 pounds of the pesticide on about 500,000 acres of forests, to prevent third-year defoliation, and to make sure that the infestation did not spread any further.

The EPA sent a team of investigators to take a look at the area and they returned with the finding that the egg masses were contaminated with virus—albeit at a fairly low level—and their report said that there was every expectation that the population would collapse without the use of DDT. They did, however, allow the experimental use of four chemical alternatives to DDT—Zectran, Dylox, Sevin-4-oil, and Bioethanomethrin—and the testing of a polyhedrosis virus and the bacterium *Bacillus thuringiensis* against the caterpillars.

The virus infection has not worked. The moth spread to an area about twice that of the original infestation. There seems to be no explanation of why the virus failed to do its work, except for the possibility that it may not have been present in sufficiently high density to wipe out the population. As for the insecticides, all were moderately effective but not as good as DDT, and the microbial agents proved to be promising but they will not be available in sufficient quantities for use in a large outbreak.

An extensive survey is now being undertaken to examine the egg masses that have just been laid, to try to predict the course of the infestation next year. Some of the eggs will be hatched in laboratories next March, and that should give some indication of the chances of virus infection wiping the insects out next spring. If it seems that the population will collapse from virus infection, the EPA could again deny the use of DDT. But the repercussions of another wrong prediction are obvious. Alternatively, the agency could agree to the use of DDT to prevent final-year defoliation of the infested trees, but that would lay it open to charges from environmentalists that DDT was used in a situation that did not warrant it. Moreover, there is a fear that if the DDT ban is lifted in this case, the floodgates would be opened for special pleas in countless other cases.

The EPA has, in fact, already granted exemption from the DDT ban this year for the spraying of pea crops in Washington to kill pea leaf weevil. That decision was taken because no other pesticide was available, and it was used in a region where there is little water runoff or likelihood of damage to wildlife. Although the exemption from the

ban has not sparked off a rash of other formal requests to use DDT, a parade of witnesses before the House Agriculture Committee last week told of numerous cases in which they would love to break out the DDT spray.

The hearings themselves were concerned with a bill introduced by Congressman Mike McCormack, whose constituents in the state of Washington are up in arms about the EPA's handling of the matter. The bill would essentially force the administrator of the EPA to approve use of DDT whenever the Secretary of Agriculture requests it. In other words, it would strip the EPA of its control over DDT. Although the committee members who bothered to attend the hearings last week are in strong sympathy with the legislation—it would, after all, give the Agriculture Department, over which they are supposed to preside, more power—its chances of survival if it ever reaches the floor of the House are considered slim. It is unlikely that many congressmen would be willing to put such a sensitive matter back in the hands of a department so closely allied with the industry concerned.

If last week's hearings demonstrated one thing, it was that the emotion that surrounded the DDT debate has by no means diminished since the pesticide was banned. The committee room echoed to all the familiar arguments, emotive phrases and accusations of scientific malpractice that characterised the extensive public hearings on the matter last year. At least the committee staff showed what they thought of the proceedings. They spent most of the time reading magazines and newspapers while witnesses on both sides of the debate rehearsed their arguments and congressmen asked a spate of naive questions.

TECHNOLOGY ASSESSMENT

Some Cash at Last

by our Washington Correspondent
THE Office of Technology Assessment (OTA) is in business at last, more than a year after Congress decided to set it up. The office, which will conduct analyses and offer advice to the legislative branch of the federal government on issues involving science and technology, has been given \$2 million to spend in the 8 months that now remain in the 1974 fiscal year. The money, which was contained in the Legislative Appropriations Bill, was delayed by a fight over an entirely unrelated matter (see *Nature*, 245, 284; 1973), but the holdup prevented the OTA from hiring staff, appointing a director or doing any work—in other words, the office existed in name only.

Although \$2 million is a good deal less than the \$5 million that the OTA's

backers were requesting for its first year of operation, the funds will allow a start to be made soon on a few specific problems, which will be tackled by *ad hoc* panels. The first move, however, will be for the OTA Board, which consists of six senators and six congressmen, and which is chaired by Senator Edward M. Kennedy, to appoint a full-time director for the office. Emilio Q. Daddario, a former congressman who was largely responsible for developing the legislation that led to the setting up of OTA, is expected to get the job early next month.

MARINER 10

Go for Take-off

by our Cosmology Correspondent

THE first spacecraft to use the gravity of one planet to help it on its way to another is due for launch on or about November 3. Mariner 10 will swing past Venus and on to Mercury, where the first encounter will take place around March 29, 1974. The spacecraft will, if all goes well, remain in a circum-solar orbit so that further encounters with Mercury will take place on September 22 and at roughly six-monthly intervals thereafter. The spacecraft should still be functioning for the second encounter, at least.

On the face of things the Venus observations form the more interesting part of the mission, since enough is now known about Venus to hint at a complex planet with slow retrograde spin, extensive cratering, and an active atmosphere. But so little is known about Mercury that any information from Mariner 10 will be invaluable. And at least Mercury is not obscured by cloud; two television cameras equipped with 150-cm Cassegrain telescopes will produce resolution of 1.5 km for the full coverage of the planet and 100 m for selected areas.

Apart from the television cameras Mariner 10 will carry six scientific experiments, including an infrared radiometer, an extreme ultraviolet experiment, a 'radio science' experiment, a magnetometer and two experiments to measure the solar wind (particularly inside the orbit of Venus) and the galactic cosmic radiation. The infrared experiments will, of course, provide estimates of the surface temperatures of both planets. On Mercury large temperature variations are to be expected, for a thin atmosphere (if there is one at all, a matter which should be settled by the ultraviolet measurements) will allow extreme heating of the side exposed to the Sun. And the relatively slow rate of rotation (once every 58.6 days) means that the night side can lose large quantities of heat by radiation.

NEWS AND VIEWS

Does Zodiacal Light Intensity Vary?

ZODIACAL light is the term used to describe the faint cones of light that extend away from the Sun in the ecliptic plane, or zodiac, of the sky. It is visible to the naked eye and can be best seen from a high mountain in the tropics where it appears in the western sky for an hour or so after the end of evening twilight, or in the east for a similar period before the beginning of dawn. The narrow cone of light stands up above the horizon and reaches about halfway to the zenith. It shares the apparent diurnal motion of the sky and during the night sinks below the western horizon until, two hours or so after its first appearance at the end of twilight, only the faint upper portions remain visible. The cone is widest at the base, where it is of comparable intensity to the Milky Way, and narrows and grows fainter towards its apex. Studies of the spectrum of this light indicate that it is caused by sunlight scattered from small, solid interplanetary dust particles. These dust particles pervade the entire Solar System and by scattering sunlight they produce about one-third of the total light shining on the Earth's surface on a moonless night (the remainder is due to airglow and background starlight). Again using spectroscopic observations the Doppler shift of several of the Fraunhofer lines in the spectrum of the light can be measured. Radial velocities determined from these observations indicate that essentially all the dust particles are in direct orbits around the Sun.

It has been deduced from polarisation observations that the zodiacal light could be produced either by many very small particles (submicron) or by fewer larger particles having rough surfaces. The similarity between the spectra of zodiacal light and Sun light supports the latter hypothesis and it is now generally believed that most of the light comes from particles smaller than $10\ \mu\text{m}$ (masses less than $10^{-9}\ \text{g}$). Over a period of about 10,000 years these particles will be dragged into the Sun by the Poynting-Robertson effect, whereby the particles lose angular momentum due to the uniform re-radiation of adsorbed solar radiation. So if the dust cloud is in a steady-state condition there must be a continual source of small particles. The exact nature of this source is still in some doubt because the dust introduced must not only be of the right size but it must also be in the right place in the Solar System. A considerable fraction will come from the breakup of cometary nuclei as they pass close to the Sun and this process leads to the formation of streams of dust particles around the orbit of the comet. These dust streams then decay into the Solar System dust cloud. Asteroidal collisions could produce sufficient dust outside the Earth's orbit, which would slowly spiral into the Sun due to the Poynting-Robertson effect. Lunar ejecta produced by meteorite impact have also been suggested.

One way of deciding between these different possibilities is to investigate the temporal constancy of zodiacal light. Continual changes in the brightness as a function of, for example, the Earth's position around its orbit could indicate that the dust cloud was not equidense but was made up of a collection of individual toroidal particle

streams. Constant brightness could, on the other hand, indicate that asteroidal debris was the main component of the cloud and/or that the meteor streams only contain large particles (mass greater than $10^{-6}\ \text{g}$) which only over a period of about 10,000 years break up into smaller zodiacal dust particles and diffuse into the cloud. A third possibility is that new streams do contain many small particles but that these are perturbed from the stream orbit very quickly. It has also been suggested that the dust does not originate in either comets or asteroids but is just an extension of the outer solar corona and has a common physical origin. These last two suggestions would both give constant brightness.

Results of a satellite-borne experiment to measure the brightness of zodiacal light (6530 Å) as a function of ecliptic latitude and the position of the Earth around its orbit are set out on page 26 of this issue of *Nature*. The satellite used, D2A 'Tournesol', was in a low altitude orbit and reaching a maximum height of 700 km above the Earth's surface. Levasseur and Blamont of the Service d'Aéronomie du Centre National de la Recherche Scientifique obtain measurements of the zodiacal light intensity at 90° elongation, that is in the plane perpendicular to the Earth-Sun line, which agree very well on normal days (that is, for most solar longitudes) with previous measurements by other researchers. On certain days, however, the zodiacal light intensity increased by up to 100% above the normal value, and this increase lasted a few days. By making measurements in consecutive years Levasseur and Blamont found the variations in intensity to occur at the same positions around the Earth's orbit. They conclude that these increases were caused when the Earth passed through a meteor stream, the 100% increase on November 1971, which remained 50% above the normal level for six days around the maximum, being the result of an encounter with the Andromedid stream, a toroid of particles in space produced by the decay of comet Biela.

The April Lyrids have also been detected, and one very interesting fact emerging from the observations is that width of the stream of small dust particles detected by the zodiacal light observations appears to be twice as wide as the stream of much larger (mass $\sim 10^{-1}\ \text{g}$) particles observed as visual meteors. Levasseur and Blamont conclude that the cloud of zodiacal dust particles is not uniform but consists of a collection of streams of particles, created by the decay of recent comets; these streams have a relatively short lifetime and diffuse into a more general background on account of the Poynting-Robertson effect. Interparticle collisions and planetary perturbations can also cause this stream decay.

Interestingly, Alexander, Arthur, Bohn and Smith (*Space Res.*, **13**; 1973) have also detected increases of up to 100% in the density of picogram particles, in this case in selenocentric space, by using microphone sensors on board the satellites Lunar Explorer 35 and OGO III. These increases were found to coincide with the major meteor showers.

A slightly cautionary note, however, must be intro-

duced. Sparrow and Ney (*Science, N.Y.*, **181**, 438; 1973), who have been measuring the intensity of the zodiacal light for four years from the satellite OSO-5, have failed to detect any temporal variations in the brightness or polarisation and have concluded that this variation if present at all is less than $\pm 10\%$ of the mean value. Sparrow and Ney discount all the many previous observations which purport to show correlations between zodiacal light intensity and solar surface brightness, magnetic storms, lunar phases, the position of Comet Encke, the Earth's position around its orbit and the eleven-year solar activity cycle.

At present these contrasting observations make the choice of a hypothesis to explain the origin of the zodiacal dust cloud very difficult. D. W. H.

New Look at Chromatin

IN the past, attempts to relate the structure of chromatin to its function have lacked incisiveness because it has not proved possible to test directly for the expression of a given gene. It can be argued, for example, that looking at the physical and chemical properties of chromatin or the rates of transcription *in vitro* from chromatin templates does not give conclusive results since they fail to establish a direct relationship between the observed phenomena and specific gene expression.

Because of this criticism some workers have tried to analyse the RNA transcribed *in vitro* from chromatin by hybridising it to homologous DNA in conditions of RNA excess. In this type of analysis increasing concentrations of RNA are hybridised to a fixed quantity of DNA until a saturation level is reached. From this figure the fraction of active DNA in chromatin was estimated and it was concluded that only certain of the DNA sequences in chromatin are transcribed. The same type of analysis has been used to compare the sequences of two RNA populations by comparative hybridisation. In some of these studies the RNAs transcribed from different chromatin templates were found to contain markedly different types of sequences (for example, Paul and Gilmour, *J. molec. Biol.*, **34**, 305; 1968). It was argued that the template activity of DNA in chromatin is restricted in such a way that only tissue specific sequences are transcribed and that this provides the basis for the observed differences.

As knowledge of the complexity of eukaryotic DNA developed it became apparent that such hybridisation studies only detect RNA sequences which are transcribed from the repetitive fraction of the DNA. Recent studies of Harrison *et al.* (*Nature*, **239**, 219; 1972) and Bishop *et al.* (*Nature*, **235**, 231; 1972; and *Nature new Biol.*, **241**, 204; 1973) have shown that a tissue specific mRNA, globin mRNA, is transcribed from the unique DNA fraction. If this is generally true for most other mRNAs, then RNA excess hybridisation studies will not reveal differences in messenger sequences. The fact that tissue specific differences were detected within the repetitive DNA fraction is therefore intriguing.

A new approach to the analysis of chromatin function is now available as a result of the recent demonstration that globin mRNA can serve as template for the RNA dependent DNA polymerase (reverse transcriptase) from avian myeloblastosis virus to give a complementary DNA

(cDNA) copy of globin mRNA. Several workers have devised kinetic analyses in which RNA populations can be tested for the presence of globin mRNA by hybridisation to cDNA. When applied to the RNA transcribed *in vitro* from chromatin this test provides a far more exact analysis.

At this year's Cold Spring Harbor Symposium two groups of workers reported studies in which RNA transcribed by *Escherichia coli* polymerase from haemopoietic chromatin was examined in this way. Gilmour and Paul of the Beatson Institute for Cancer Research, Glasgow, compared the RNAs transcribed from mouse foetal liver (a haemopoietic tissue) and mouse brain chromatin by hybridising the RNAs to cDNA complementary to mouse globin mRNA. The results show that RNA transcribed from foetal liver chromatin contains globin mRNA sequences (1–2 parts in 10^5), whereas RNA transcribed from brain chromatin contains no detectable sequences. Axel *et al.* at the National Institutes of Health, Bethesda (see *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2029; 1973), made a similar comparison between RNAs transcribed from duck reticulocyte and duck liver chromatin and found that 7 parts in 10^5 of reticulocyte chromatin RNA corresponded to duck globin mRNA whereas liver RNA contained no globin sequences.

Both groups eliminated the possibility that the globin mRNA had arisen by contamination with cellular RNA rather than by *de novo* synthesis, by incubating the haemopoietic chromatin in the absence of polymerase and finding, after adding carrier RNA, no material hybridising to cDNA. Furthermore, Gilmour and Paul isolated the hybrid formed between cDNA and RNA transcribed from mouse foetal liver chromatin on CsCl gradients. By labelling the RNA with ^{32}P it was possible to estimate that most of the hybridisable RNA arose by polymerase action and not cellular contamination. These results demonstrate that the transcription of DNA in chromatin is restricted in a very specific way and that this property survives the chromatin isolation procedures. Moreover, the findings show that bacterial polymerase can transcribe the globin gene and that the sequences transcribed by it are subject to a control mechanism. The conclusions then are very similar to those obtained from the earlier experiments in which RNA excess hybridisation was used.

The finding that tissue specific differences exist in the RNA transcribed from reiterated DNA sequences may be of considerable importance. By annealing RNA transcribed from chromatin in conditions of DNA excess, Axel *et al.* demonstrated that most of the RNA is transcribed from unique DNA sequences and only a small part comes from repetitive DNA. A similar analysis of *in vivo* nuclear RNA by Melli *et al.* (*Nature*, **231**, 8; 1971) gives an analogous result. It is now becoming clear that there is a relationship between unique and repetitive sequences both in RNA and DNA. Firtel and Lodish (*J. molec. Biol.*, **79**, 295; 1973) examined the nuclear mRNA precursor of *Dictyostelium* and found that about 25% of the sequences are transcribed from repetitive DNA, the remainder being unique. Most of the repetitive sequence, which is at the 5' end of the RNA, is removed during nuclear processing; however, a small portion accompanies the RNA into the cytoplasm. Similarly, Dina *et al.* (*Nature new Biol.*, **242**, 101; 1973) found that mRNA from the polysomes of *Xenopus* embryos contain two types of sequence, one which hybridises to the repetitive DNA and the other which is complementary to unique

DNA. For *Xenopus*, Davidson *et al.* (*J. molec. Biol.*, **77**, 1; 1973) have presented evidence that the types of sequence are interspersed throughout the genome and they propose that 200–400 bases of unique sequence alternate with longer unique sequences of average length 600–900 nucleotides, large enough to accommodate a single gene.

It is attractive to speculate that the short repeated sequences represent control elements which regulate the transcription of adjacent unique sequences. As already noted some of these repeated sequences are transcribed and indeed tissue specificity in the repetitive sequences of *in vivo* RNA is demonstrable in the earlier experiments of Shearer and McCarthy (*Biochemistry*, **6**, 283; 1967). The possibility that there is a degree of specificity in the repetitive RNA sequences from different tissues provides an explanation of why RNA excess experiments with chromatin-primed RNAs show tissue specificity. Hence within the repetitive sequences there may be broad specificities which reflect in a general way the specific mRNA sequences of a given tissue. Obviously because of the relatedness of repetitive sequences and the present uncertainty about their function this is not a particularly definitive parameter of gene expression. Hybridisation to cDNA complementary for a known mRNA clearly offers the most exact method available.

At the Cold Spring Harbor Symposium, the Glasgow group also reported results which indicated how this new approach might be used to identify molecules concerned with the restriction of chromatin. It was found that when the structure of foetal liver chromatin is disrupted by dissociating the DNA and proteins in high salt-urea solutions and then the components reassociated by slowly dialysing away the salt, the resulting chromatin still serves as a template for globin mRNA sequences. This means that the specific way in which chromatin is assembled is an inherent property of the components themselves. Which are the molecules responsible for directing this self-assembly?

A preliminary investigation implicates molecules within the non-histone or acidic protein fraction of chromatin. Non-histone proteins from foetal liver were isolated free of DNA and histones on hydroxy-

apatite columns. This material was allowed to associate with mouse brain chromatin by the reconstitution process already described. It could be shown that globin mRNA sequences which are normally absent from brain chromatin transcripts are now detected in the RNA transcribed from brain chromatin reconstituted with liver non-histone proteins. This suggests that there is a component of liver non-histone which is responsible for controlling the expression of the globin gene.

The isolation and handling of non-histone proteins have now reached a stage where virtually pure fractions of individual components can be obtained. Although some of these are probably chromatin-associated enzymes or structural proteins rather than control elements, it is now possible for the first time to make this distinction and, hopefully by narrowing down the field, ultimately to identify the responsible molecules.

S. G.

MEDICAL RADIOLOGY

Benefits and Risks

from a Correspondent

PATIENTS undergoing medical radiology are subject to small, but not negligible, genetic and somatic hazards. If the risks and benefits could be quantified, a cost/benefit study could be undertaken and measures to reduce the detriment could be assessed realistically against the likely gain. This was the

main theme of a conference organised by the Society for Radiological Protection in Birmingham on October 9.

The evaluation of the risks involves four stages. First, the radiation doses to individual organs are determined by direct measurement or otherwise; next, for each organ the mean dose to the population is assessed, taking into account the number of patients involved, their age distribution and other factors; third, the biological effect of a given organ dose is estimated; finally, a monetary value is attached to a given detriment.

The radiation doses involved are known reasonably well. By far the largest contributor is diagnostic radiology and this field was reviewed by S. B. Osborn (King's College Hospital, London). The evidence is derived mainly from the 1960 and 1966 reports of the Adrian Committee. The mean genetically significant dose (GSD) from diagnostic X rays to the whole population of the United Kingdom is about 15 mrad yr⁻¹, and the annual mean bone marrow dose is about 32 mrad. In other countries the GSD ranges from 1 to 50 mrad depending on the amount of radiology undertaken and the techniques used. Osborn pointed out that very large variations occur from hospital to hospital and the United Kingdom detriment could be reduced considerably if all institutions reached the standard of the best. This could not, however, be achieved without additional expenditure.

The contribution of radiotherapy to the mean annual GSD is about 5 mrad, whereas that from radioisotope tests is currently about ten times lower. The use of unsealed radioisotopes, however,

Conditions for Restriction in *E. coli*

MANY strains of *Escherichia coli* recognise the DNA of other strains and of phage. Recognition is followed by hydrolysis of the invading DNA, with a strain-specific endonuclease, unless the DNA is modified (by glucosylation and methylation) or mutated—presumably at the recognition site (SB site). A DNA duplex with only one strand modified is protected from degradation. Some experiments by Hartman and Zinder, of the Rockefeller University of New York, suggested that in some FI phage crosses of DNA duplexes only one strand is mutated at an SB site. They wondered how this mutant hybrid would compare with a modified hybrid when challenged with restriction enzymes and so, together with their colleagues Vovis and Horiusha, designed experiments to tackle the problem. Next Wednesday's *Nature New Biology* (November 7) contains an account of this work.

DNA heteroduplexes were made

in vitro by annealing the wild-type DNA of phage FI with alkali denatured RFIII/PL (that is, full length, linear, double stranded DNA enzymatically prepared from the replicating form of the virus-RFI, with restriction enzyme PL). The resulting heteroduplex, RFII, is circular, double stranded with at least one single strand nick.

The phage FI has two sites SB₁ and SB₂ which confer susceptibility to *E. coli* B restriction. By making heteroduplexes in which one or other of the DNA strands was modified or mutated at the SB sites, and testing for restriction with *E. coli* enzyme B, it was possible to confirm that both modification and mutation of either strand of a DNA duplex at the SB site protect the molecule from restriction.

The authors were not able to determine whether SB mutant hybrids can be modified *in vivo* or *in vitro*, but they are at present working on this question.

is doubling every 3 yr. R. E. Ellis (University of Leeds) has found (ICRP Publication 17) that most individual radionuclide investigations give rise to somatic and genetic doses to patients comparable with those from X-ray examinations, but there are important exceptions and he proposed a set of maximum organ doses (for example, 5 rad for non-pregnant adults) for these tests.

The assessment of the biological effect of small radiation doses was discussed by J. Rotblat (St Bartholomew's Hospital Medical College, London). This is the most difficult step of all, since the effects must be extrapolated from the observed results of irradiation at relatively high dose levels. In the absence of direct evidence, a linear extrapolation is often assumed, but Rotblat thought that this may sometimes underestimate the effect. The evidence from Hiroshima indicates an annual death rate from radiation-induced cancer of all types of $\sim 6/10^6$ adults per rad. For young persons, however, the rate per rad seems to be several times higher, and it is still higher for embryos. For continuing exposure the induction rate depends on the latent period and the number of remaining years of life during which cancer may become manifest. Rotblat concluded that medical uses of radiation in the United Kingdom may lead to an annual cancer rate of 150–750, with perhaps a further 25–600 diseases or abnormalities of genetic origin.

G. Webb (National Radiological Protection Board) discussed the methodology and potential uses of a cost/benefit study in this field. Although a rigorous analysis is not yet feasible, useful results can still be obtained. The overall cost to society is reduced if patient doses are held at the lowest level consistent with a high rate of successful diagnosis and treatments. According to ICRP Report 22 the cost of the detriment associated with 1 man-rad is £10–£100. If the mean GSD to the UK population is 20 mrad, the annual cost of the genetic detriment alone is £10⁷–10⁸, a significant fraction of the total cost of the National Health Service ($\sim £2 \times 10^9$ yr⁻¹). Webb concluded that the British people can afford to invest a little more each year in the health of future generations.

PHARMACEUTICS

Safety of Contraceptives

from a Correspondent

A MEETING organised by the World Health Organisation was held in Geneva from September 16 to 20 to discuss the use of pharmacological models in the assessment of toxicity and side effects of

fertility regulating agents. The meeting reflected the uncertainty felt by many involved both in the development of new fertility regulating agents and the continued use of presently available ones concerning the assessment and monitoring of toxicity and side effects. A major part of the deliberations was concerned with the extent to which studies performed on animals could be extrapolated to man. The usefulness of the various tests of toxicity and anti-fertility carried out in a number of species was reviewed. To have predictive value these tests should be performed in species in which the reproductive mechanisms, toxic reactions and metabolic disposition of the agents are similar to those in humans. Because of the wide variability of these parameters in different species a number of different species would probably have to be used, an obviously unsatisfactory solution.

If animal models are unsuitable for assessing toxicity, the careful monitoring of subjects using the regulatory agents is of extreme importance and this again raises special problems. These agents are used by normal healthy women over long periods of time and often with little medical supervision. Side effects which occur with a high frequency would be quickly noted but those which occur with a low frequency would be much more difficult to detect. It is these low frequency side effects which are difficult, if not impossible, to detect in animals. As expected, the question of carcinogenicity of presently used hormonal contraceptives provoked much discussion, although those who suggest on theoretical grounds that the oral contraceptives might be carcinogenic in humans have difficulty in finding evidence to support their views. No satisfactory model is presently available to test for carcinogenicity. In considering contraceptive drugs the return of fertility at the end of the treatment period has to be ascertained. The possibility that women might become pregnant while taking, or shortly after having taken, the drug necessitates screening for teratogenic and mutagenic effects.

The requirements for toxicity testing of anti-fertility agents were discussed by representatives of the national drug regulatory agencies with particular consideration to the possibility of standardising many of the requirements on an international basis. These requirements must change in the light of new knowledge. The advantages and disadvantages of methods available for monitoring side effects in humans were also reviewed. The evaluation of reported side effects is complicated by the incidence of placebo reactions and these have not been taken into account in many trials. In addition, variations in response of women of different ethnic

origins to the same contraceptive drug are important. The special requirements for toxicity testing of long acting and of post-coital contraceptives, of spermicides, of various types of intrauterine devices and of the prostaglandins, all of which pose special problems, were also discussed.

It would not be advantageous to increase the amount of animal toxicity testing since this would increase the cost of research and development and, in the opinion of one speaker, "lead to a decreased interest on the part of the pharmaceutical industry to develop new agents. If this were to happen it may ultimately be more disastrous for the survival of large parts of the world population than the limited risk of even serious adverse effects of oral contraceptive preparations". It is hoped that advances in contraceptive technology would lead to a decrease in the amount of toxicity testing that needs to be carried out and hopefully shorten the time between animal testing and preliminary evaluation in humans.

Not unexpectedly, at the end one felt many questions remained unanswered, more research needed to be done, present methodology required improvement and many more discussions were imperative.

CELL BIOLOGY

Control of Proliferation

from a Correspondent

THE sixth meeting of the European Study Group for Cell Proliferation, which was held in Moscow from September 25 to 28 at the Institute of Molecular Biology of the Academy of Sciences of the USSR, took the general title "Molecular Mechanisms and the Control of Cell Proliferation".

Among the invited participants Professor J. M. Mitchison (University of Edinburgh) reviewed the concept of cell cycle markers, within which he included any detectable event which is associated with a localised region of the intermitotic period. Markers fall into three categories: morphological events such as mitosis or abrupt changes in the structure of the cell surface; biochemical events such as the initiation of DNA synthesis or the induction of specific enzymes; transition points at which metabolic inhibitors either begin or cease to be effective. The markers that are available within any one proliferating cell system indicate detectable points in the sequence of gene expression through the cell cycle. In some cases the blocking of one event in this sequence may suppress later events. Other examples can be found in which the sequence of events that make up the "DNA synthesis-division" cycle may to some extent be dissociated

from the sequence of the "cell growth" cycle.

At present a great deal of interest centres on the ability of proliferating cells to move into a state in which the DNA synthesis-division sequence is interrupted, as well as on their ability to respond to a suitable stimulus by continued proliferation. Yu. M. Vasiliev (Institute of Oncology, Moscow) reviewed the evidence for this in cultures of normal and neoplastic cells, which can enter a quiescent state either as a result of medium deprivation or confluency and renew proliferation following mechanical damage, the addition of fresh serum or treatment with a variety of chemical agents.

An important contribution to understanding of the nature of contact inhibition of growth came through the work of A. Zetterberg (Karolinska Institute, Stockholm) who has studied the approach to confluency in cultures of mouse kidney epithelial cells. When the cells were grown at low cell densities on a solid support, a thin web of cytoplasm formed around them, clearly demonstrable in the interference microscope, to which the term 'lamellar cytoplasm' was given. Detailed studies of the relationship between cell surface area and thymidine-labelling index led to the hypothesis that the cellular proliferation rate is largely determined by the area of the lamellar cytoplasm, with some dependence also on the nutrient quality of the medium. The biochemical changes that take place in confluent cell monolayers following stimulation by a change of medium were described by R. Baserga (Temple Medical School, Philadelphia). In cultures of normal human fibroblasts, an increase in the synthesis of non-histone chromosomal protein is detectable within 30 min of stimulation, closely followed by an increase in chromatin template activity, but neither of these early responses was found following stimulation of virus-transformed fibroblasts. The proffered papers, of which there were more than fifty, illustrated the existing wide interest in the control of cell proliferation both *in vivo* and *in vitro*, with sessions devoted to cell proliferation in haemopoietic tissues and to the possible role of chalones in tissue homeostasis.

The European Study Group for Cell Proliferation, which was formed in 1967 under the auspices of the Coordinating Committee for Human Tumour Investigations, has now gained affiliation to the European Cell Biology Organisation. Its meetings have been designed to span a wide range of interests from the biochemistry of cell proliferation, through the study of cell and tissue kinetics, to the attempt to relate cell proliferation to therapeutic response in cancer. The next meeting will be in Amsterdam in the spring of 1975.

CELL BIOLOGY

Proteases and Growth

from a Correspondent

THE intriguing correlation between proteolytic activity, high agglutinability in the presence of plant lectins, and cell growth has been studied extensively in recent years. Transformed cells are readily agglutinable by lectins and continue to grow in conditions which arrest normal cells. Normal cells are much less readily agglutinated but on treatment with proteases they become agglutinable and, it is claimed, start growing again. It has been proposed, on the basis of these observations and others, that proteases produced by transformed cells act upon the surfaces of the cells and trigger them into growth.

This simple picture has now been questioned by Glynn, Thrash and Cunningham who report (*Proc. natn. Acad. Sci., U.S.A.*, **70**, 2676; 1973) that pronase treatment of 3T3 cells which have stopped growing renders the cells agglutinable by lectins but does not start them growing again. The increase in agglutinability, whatever it means, is in agreement with results from a number of laboratories, notably that of Burger (see *Fed. Proc.*, **32**, 91; 1973, for review) and Sachs, but the failure to stimulate

growth suggests that the alteration in cell surface produced by pronase and detected by lectins is not sufficient to trigger growth as has been suggested. Earlier reports have claimed that proteases, including pronase, when applied to cells, including 3T3, do start them growing (Burger, *Nature*, **227**, 170; 1970; Sefton and Rubin, *Nature*, **227**, 843; 1970).

There seems to be a direct conflict of results here. One might argue that Glynn *et al.* had damaged their cells so that they could no longer respond to growth stimuli. They show, however, that serum added after the pronase treatment is still effective in growth stimulation, although the response was not quite so marked as when the pronase treatment was omitted. They also state that the treated cells still respond to cortisol, another growth initiator.

The solution to this conflict could be in minor differences in procedure: perhaps the media of Burger and of Sefton and Rubin were less completely depleted of serum factors than those of Glynn *et al.* thus allowing or assisting the cells to respond to a growth stimulus by the pronase. The answer will have to await further experiments but it is clear from the results of Glynn *et al.* that one can separate the surface changes produced by proteases from any growth stimula-

Vesicular Stomatitis Virus Cores add Poly(A)

VESICULAR stomatitis virus (VSV) is a bullet shaped, negative strand virus that infects a wide variety of animal cells. Its genome is a single large piece of RNA that acts as a template for a virion-associated polymerase. This enzyme can be unmasked *in vitro* by non-ionic detergents such as NP 40. The RNA made by detergent-treated virions is considerably smaller than the genome, and complementary to it, but similar RNA species are found to be associated with the polysomes of VSV infected cells.

The MIT group of Baltimore, Lodish and colleagues have recently shown that these RNA molecules code for authentic VSV proteins in the wheat-germ cell-free protein synthesising system, so it seems legitimate to call them mRNA. Mudd and Summers (*Virology*, **42**, 958; 1970) showed three years ago that these messages contain poly(A), but a certain confusion has arisen as to when, where and how the poly(A) gets added.

Everybody agrees that the genome lacks the poly(U) tracts which would be necessary to code for the poly(A), but Roy *et al.* (*J. Virol.*, **11**, 915; 1973) recently said that the detergent-disrupted virions fail to make any poly(A). At the same time, Galet and Prevec (*Nature new Biol.*, **243**, 200; 1973) found that cytoplasmic extracts of VSV

infected cells would make mRNA which had the poly(A) stretches. These results seem to suggest that the host cell has a cytoplasmic enzyme which can add poly(A) to a viral RNA primer. This hypothesis is probably unnecessary, however, for Villareal and Holland report some experiments with detergent-treated VSV in *Nature New Biology* next Wednesday (November 7). They found that if they purified the cores by gel-filtration on Biogel A-5M they (1) obtained considerably higher activity and (2) the mRNA produced contained poly(A).

Unlike the activity found in Vaccinia virus cores (Kates and Beeson, *J. molec. Biol.*, **50**, 19; 1970), VSV cores need all four nucleoside triphosphates to make poly(A), suggesting that a primer is required to which the poly(A) is added. The nature of the primer is unclear, however, for Villareal and Holland find that even the small pieces of RNA produced by the defective interfering 'T particles' of VSV can have normal poly(A) added to them. Nor is it clear whether the primed but template-independent addition of poly(A) is made by the RNA-dependent RNA polymerase, or whether some other component of the purified cores—perhaps the so-called NS protein—is responsible.

tion. So, if proteolytic digestion assists in removing controls involved in density dependent inhibition of growth, which is not ruled out by these results, it does not manage it by itself. This conclusion is perhaps not surprising but worth stating in any case, especially for those cell biologists who are studying the role of serum factors and hormones in growth control.

THEORETICAL CHEMISTRY

Quantum Chemical Game

from our Molecular Physics Correspondent

ALL too often the complexities of the many-electron problem in chemistry lead to a frontal, and sometimes premature, assault by computer, relieved only occasionally by the elegance of a result from group theory or elsewhere. So familiar has this pattern become that it is a pleasant surprise to be presented with a simplification that is both unusual and elegant and, in its way, outright amusing.

Such a description may justly be applied to the paper entitled "A Game Theoretic Model for the Determination of Charge and Configuration in Metal Complex Compounds" by Haberditzl and Bartel (*Chem. Phys. Lett.*, **19**, 432; 1973). The authors show how at least one stage of the, in principle enormously complicated, problem of calculating the distribution of electrons between the central ion of a metal complex and its neighbours can be reduced to a simple exercise in the theory of games, the ion and its neighbours 'competing', as it were, for the electrons available. The type of idealised game involved proves to be quite a simple version of the 'two-person zero-sum' game in which two players seek an optimal course of action to maximise their chance of gain from a fixed premium. The various 'pure strategies' in the game correspond to suitable wave functions for the ion and the ligand system, and the 'payoff matrix' gives the hypothetical gain of electrons by the central ion for differing combinations, with due allowance for symmetry.

Of course the elements in the latter must be calculated by whatever means are to hand—inevitably some form of perturbation theory—and it is here that the full difficulty and scope for ingenuity in the problem very much remain. Yet, once the 'payoff' conditions have been specified, the optimal strategy for the central ion can be computed in a very simple manner and, moreover, in a single step (with considerable advantage over the laborious and somewhat fickle iterative methods used up to now).

Specialists will no doubt be quick to delimit the scope and practicality of the method, but it seems entirely possible that it will find applications to 'competi-

JUPITER

Plea for Observations

by our Cosmology Correspondent

PIONEER 10 is now rapidly approaching Jupiter, and in a recent issue of *Icarus* (**20**, 52; 1973) Coffeen, of the University of Arizona, puts in a plea for ground based observations to coincide with those of the probe. The period of particular interest is from November 28 to December 10, and there is a need for ground based photography, spectroscopy, photometry and polarimetry; during that period, Jupiter will be at a declination of $18^{\circ}.8$, with phase angle 9° , 4.1 h east of the Sun, so that southern latitude observations at around the time of evening twilight will be most effective.

tive' situations other than that between a metal ion and a ligand system. At the same time no one should go out of their way (as the authors conspicuously refrain from doing) to see anthropomorphic subtleties in the interatomic battle for electrons. The game-theory insight reveals a curious and perhaps far reaching new minimum principle, separates a messy calculation into two usefully distinct stages, and provides an illuminating mapping of one computational problem into a better known one. Quantum chemistry, perhaps of all theoretical sciences one of the most liable to be seen as a confusion of woods and trees, can certainly do with more adventurous touches of this kind.

PETROLOGY

Kimberlite Conference

from a Correspondent

ALTHOUGH kimberlites provide a more thorough sampling of the upper mantle than any other type of magmatic rock, they have never before formed the focal point of a major international conference. This was largely redressed when the first international conference on kimberlites was held, appropriately in South Africa, at the University of Cape Town from September 23–29.

In the session on kimberlite geology, descriptions were given of less well-known kimberlite provinces in North America, Greenland and South Africa, and M. Bardet (Bureau de Recherches Géologiques et Minières, Orléans) described kimberlites from West Africa that lack the normal kimberlite 'indicator' minerals (pyrope, picroilmenite) and suggested that many of these atypical kimberlites are yet to be discovered, having been missed by current prospect-

ing techniques. Although there was general agreement on an explosive origin of kimberlite diatremes, there was considerable debate as to whether the explosion mechanism results from high pressure gases within the kimberlite magma or is due to super-heating of groundwater by the ascending magma. In this context, the D/H and $^{18}\text{O}/^{16}\text{O}$ studies by S. M. F. Sheppard (Scottish Universities Research Reactor Centre, East Kilbride) indicated the involvement of warm groundwater in the formation of some diatreme kimberlites. Micro-probe analyses of the hitherto neglected groundmass minerals in the kimberlite matrix have now provided valuable information on the physical conditions prevailing during the crystallisation of the kimberlite matrix. This was exemplified by S. E. Haggerty (University of Massachusetts) whose work on the opaque phases has revealed considerable variation in the redox conditions, cooling rate and solid-solid, solid-gas and solid-liquid reactions during crystallisation. Haggerty also reported the occurrence in kimberlite of a new barium-vanadium titanate, and the first terrestrial occurrence of armalcolite.

In the sessions on xenoliths, two aspects made the most impact. Until now virtually no work has been carried out on the petrofabrics of kimberlite xenoliths, but A. Nicolas (University of Nantes) and B. Harte (University of Edinburgh) both reported a wide variety of deformation and recrystallisation features that have been imposed on these rocks within the upper mantle. The second aspect linked up strongly with the deformation studies of Nicolas. F. R. Boyd (Geophysical Laboratory, Washington) and P. H. Nixon (Department of Mines, Lesotho) showed that highly-sheared lherzolites have equilibrated at relatively high temperatures ($1,300\text{--}1,400^{\circ}\text{C}$) compared with the more normal granular lherzolites ($950\text{--}1,100^{\circ}\text{C}$), and together with the granular lherzolites could be used to infer a Cretaceous geothermal gradient; also Boyd and Nixon interpreted the shearing as due to the movement of the African Plate during the breakup of Gondwanaland.

The geochemistry of sheared lherzolites provided another focus of attention. They contain more CaO and Al_2O_3 than the granular lherzolites (and hence more potential basalt) but A. J. Erlank (University of Cape Town) showed them to be strongly depleted in both Nb and Zr. In addition N. Shimizu (Geophysical Laboratory, Washington) reported that the clinopyroxenes in the sheared lherzolites have very high K/Rb ratios (2,000–2,200), extremely high K/Cs ratios (1,000,000–3,000,000), and low $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (0.7027–0.7038). This apparent clash between the major and trace element evidence, together with the wide-

spread occurrence of sheared lherzolites, underlines the need for more data on this important rock type.

The inclusions in diamonds have already provided valuable information on the phases coexisting with diamonds. With the increased sophistication of chemical techniques even more data have been provided by these unique sources of upper mantle information. For example, H. Allsopp (Bernard Price Institute, University of the Witwatersrand) reported U/Pb ages for diamonds from the Premier Mine, and H. Fesq (NPRU, University of the Witwatersrand) was able to show, on the basis of analysis of tiny drop-like inclusions within diamonds, that the liquid from which diamond crystallises is rich not only in siderophile elements but also in carbonate and sulphur.

The genesis of kimberlite was the subject of several papers on experimental petrology. There were two opposing viewpoints: (1) that kimberlite results from fractionation of a picritic parent, and (2) that kimberlite is an incipient melt from a phlogopite-bearing parental lherzolite. In the context of the second viewpoint, several authors reported that, although in the system peridotite-H₂O the initial melt is silica-saturated, when CO₂ is the dominant vapour phase the initial melt is silica-undersaturated. In a wider context, there seemed to be general agreement among delegates that there is a genetic link between kimberlite and carbonatite.

SUPERCONDUCTIVITY

Stimulated Tunnelling

from our Condensed Matter Correspondent Granqvist and Claeson of the Chalmers University of Technology in Gothenburg report in a recent issue of *Physical Review Letters* (31, 456; 1973) that they have observed some interesting new structure in the current-voltage characteristics of superconducting tunnel junctions which may, they suggest, be attributable to the stimulated emission of phonons.

Tunnelling is a phenomenon which occurs when two conductors are separated by a very thin non-conducting barrier: according to quantum mechanics there is a finite probability that an electron approaching the barrier from one side may spontaneously appear on the other side. If a small voltage is applied across the junction, a current will flow from conductor A which is at the higher potential, because it will have a large number of electrons which can tunnel across into empty states of the same energy in conductor B, on the other side of the barrier. A current does not flow in the reverse direction because there are no empty states in A of the same energy as that

of even the highest states in B. Nature permits only one electron per quantum state so that, if no empty states of appropriate energy are available, tunnelling cannot occur.

In the case of a superconductor, there is an energy gap 2Δ separating the superconducting, paired, electron states from the normal single electron states above them. For temperatures well below that of the superconducting transition, all the superconducting states will be occupied and all the normal ones empty. Tunnelling between two superconductors cannot occur, therefore, until the applied voltage V is sufficient to raise the potential of A to the extent that some of its occupied states reach the same energy as some of the empty states above the energy gap in B, which clearly requires $eV = 2\Delta$. The characteristic of such a junction is therefore: zero electron tunnelling current i up to the so-called gap voltage $2\Delta/e$; a very rapid increase in i as V just exceeds $2\Delta/e$, because of the particularly large density of permitted quantum states on either side of the energy gap; and a slower increase in i at larger values of V as tunnelling

takes place between states far from the energy gap whose density is much the same as that when the metal is in its normal state. The structure observed by Granqvist and Claeson consists of a series of unexpected wiggles in this latter part of the characteristic, and was only seen when electrode B was made exceedingly thin, about 1 nm.

The junctions were prepared by vacuum evaporation onto a germanium coated quartz substrate, using an elaborate technique in which the final, very thin, metal film electrode was deposited *in situ* in the cryostat at 0.3 K. The inner film was always of aluminium, which could be surface oxidised to produce the necessary layer of insulator, but a number of different superconductors were chosen for use as the thin outer electrode. The equipment was designed to measure, not i , but di/dV as a function of V , so the structure was resolved as sharp peaks. These peaks were invariably found to occur at potentials which were $2\Delta_i/e$ and $4\Delta_i/e$ above that of the main peak at $(\Delta_{A1} + \Delta_i)/e$, where $2\Delta_i$ is the energy gap appropriate to the very thin outer

Cavities in the Pulsar Magnetosphere

TAKE a highly magnetised sphere called a neutron star, and rotate it rapidly. Describe the region of space surrounding it. That, in essence, is the problem of the pulsar magnetosphere.

This is a very difficult problem, as it turns out, and indeed the complexity and variability of pulsar radiation lead one to expect that an apparently very simple problem may not have a simple, static, solution. Goldreich and Julian showed that the large electric fields induced by the rotating magnetic fields would be compensated for by a dense, charged magnetosphere, co-rotating with the star out to a 'velocity of light cylinder', where the co-rotation velocity approaches the velocity of light. Their solution was for a rotation axis coincident with a dipole magnetic axis. The perpendicular case is less tractable, but is known to have many similarities. The part of the problem which has so far received most attention is the configuration of the magnetic field lines as they cross the velocity of light cylinder. A new idea by Holloway (in *Nature Physical Science* next Monday, November 5) opens up the possibility of different configurations of charge inside the magnetosphere, and also gives a possible lead into the problem of the 'drifting sub-pulses'.

Holloway shows that a cavity within a positively charged portion of the magnetosphere would be stable, in the sense that the electric field at the boundary of the cavity would prevent

positive charges re-entering it. (There are no negative charges present, since there is complete charge separation in the magnetosphere.) The cavity could be a large sheet, dividing the magnetosphere into two regions. The situation is not necessarily static, however, since it is now possible for such a cavity to divide regions with different rotation speeds. In particular, there is likely to be a region which rotates faster than the star. This is interesting, because it provides a possible location for the emission of discrete sub-pulses of radio waves. These are often observed to 'drift', occurring earlier during each main pulse; the source thus super-rotates.

Differential rotation of this type would disturb the static picture, and the cavity would tend to fill through a viscous drift of particles. But there is a powerful dynamo in action around the star, and powerful centrifugal forces near the velocity of light cylinder. There is plenty of driving power available for re-creating the cavity. Holloway suggests that the centrifugal forces are responsible for depleting the charges in the cavity, but there is no detailed analysis as yet.

There is more to the problem of the rotating sphere than appears at first sight. It will not be sufficient to obtain a static self-consistent solution of the rotating charged magnetosphere. Holloway now explains that it may contain differential rotation, cavities, viscous drift and a centrifugal wind.

electrode and Δ_{Al} that for aluminium. This behaviour was consistently followed for outer electrode materials ranging from tantalum with $2\Delta_t = 1$ meV to lead with $2\Delta_t = 2.6$ meV, and in each case the structure diminished and finally disappeared as the outer electrode was made thicker.

Granqvist and Claesson suggest that these phenomena arise from the stimulated emission of phonons (quanta of lattice vibrational energy) in the outer electrode. The tunnelling electrons must arrive there with an energy $eV - \Delta_{Al} - \Delta_t$ above the top of its energy gap, and it is known that most of these first relax to states just above the gap through emission of so-called relaxation phonons of energy $eV - \Delta_{Al} - \Delta_t$, subsequently falling into paired superconducting states below the gap with emission of recombination phonons with energy $2\Delta_t$. If V is such that the recombination and relaxation phonons have the same energy, then there is a probability that the relaxation process will be stimulated by the $2\Delta_t$ phonons to occur faster than it otherwise would, so that, because a larger number of empty states will therefore be available into which tunnelling can occur, the current will seem to be anomalously large for that particular value of V , leading to a peak in di/dV . The required value of V is clearly $(\Delta_{Al} + 3\Delta_t)/e$, so one would expect a peak in di/dV , due to this effect, at $2\Delta_t/e$ above the gap voltage of $(\Delta_{Al} + \Delta_t)/e$, in agreement with experiment. Processes involving stimulated emission by two or more $2\Delta_t$ phonons should, by a similar argument, lead to further peaks at $4\Delta_t/e$, $6\Delta_t/e$ and so on, which is again consistent with experiment, although peaks beyond the one at $4\Delta_t/e$ are apparently too small to be observed.

The authors believe that these effects have not been observed previously because other workers were unable to prepare such thin outer electrodes: the thinner the electrode, the greater the density of recombination phonons, and so the larger becomes the probability of stimulated emission.

The density of these phonons must also be directly related to the recombination rate, so one would expect the magnitude of the new structure to depend markedly on the magnitude of the tunnelling current. A simple test of the theory would therefore be to repeat the experiment with junctions of different impedance, which can easily be arranged by varying the thickness of the oxide layer. The failure of a preliminary experiment of this nature to yield the anticipated result leads one to suspect that a complete explanation of Granqvist and Claesson's structure may turn out to be somewhat more complicated than they have suggested.

NUCLEAR PHYSICS

Resonance Transfer

from our Nuclear Structure Correspondent

WHEN an ion collides with an atom, the probability of electron transfer depends on the time they spend near each other (the collision time) and on the transfer time itself. If these times are similar, the transfer of one electron is most likely. If the collision time is twice the transfer time the electron can be transferred from the atom to the ion and back again, and so on. Since the collision time is inversely proportional to the relative velocity of the two atoms, the probability that an electron will be transferred from the atom to the ion attains peak values at a series of energies corresponding to one, three, five . . . transfers of the electron. A simple analysis shows that the cross section is of the form

$$\sigma(E) = A(E) + B(E)\cos^2(bE^{-1/2})$$

where $A(E)$ and $B(E)$ depend on the incident energy E and b is a constant. Measurements of electron transfer have been made by Ziembra and Everhart (*Phys. Rev. Lett.*, **2**, 299; 1959) that confirm this formula with surprising accuracy.

The simple theory works very well for the transfer of electrons between atoms because the electron is very light compared with the atoms themselves. If the transferred particle is comparable in mass to the colliding particles much of the simplicity is lost because of the increasing importance of recoil effects.

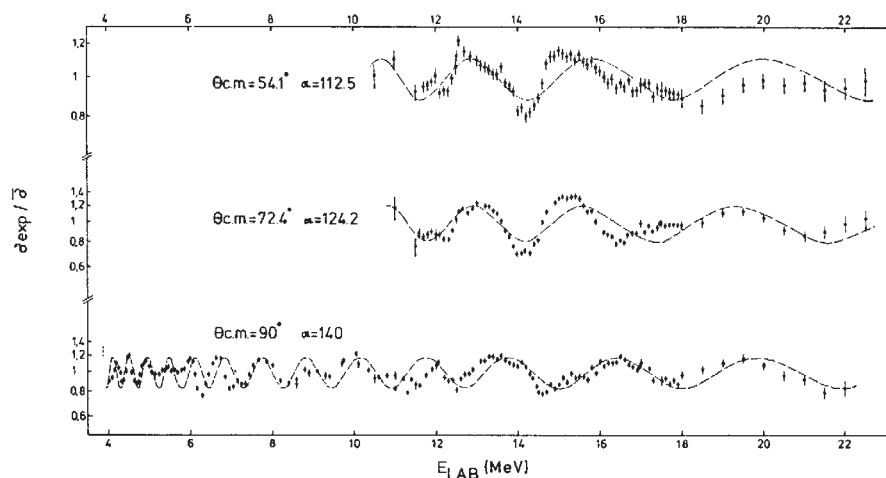
Resonant transfer processes, as they are called, have been looked for in nuclear reactions, and in particular it has been conjectured that they might be observable in neutron-proton collisions as multiple pion exchange or in heavy ion reactions as nucleon or cluster

exchange. Some indications of the characteristic signature of resonant transfer, a cross section following the formulae outlined here, have been found from time to time, but have not been substantiated by further work.

Recently a more convincing example of resonant transfer has been found in a study by Kelleter, Hrehuss and Mayer-Börcke (*Nucl. Phys.*, **A210**, 502; 1973) of the scattering of alpha particles by ${}^7\text{Li}$. The cross section of this reaction shows a rather irregular dependence on energy, as might be expected for such nuclei, but if it is plotted relative to the energy-averaged cross section an oscillatory behaviour is apparent, as shown in the figure. In particular, it shows an increase of 'wavelength' with energy very similar to that given by the resonant transfer, with small deviations readily attributable to the neglect of recoil and other effects in the simple theory. Since ${}^7\text{Li}$ can be considered as formed by a triton bound to an α particle, this oscillatory component of the reaction may be interpreted as due to transfer of a triton between the incident α -particle and the α particle in ${}^7\text{Li}$.

This interpretation is strengthened by an estimate of the parameter b , which is approximately given by $E_{\text{ex}}t_{\text{int}}/\hbar$, where E_{ex} is the average exchange energy and t_{int} the interaction time. The values of b obtained in this way are in qualitative agreement with those found by fitting the resonant transfer formula to the measured cross sections.

Further work needs to be done to develop a detailed theory of the process, taking into account the recoil effects and the presence of other reaction channels, and it would be useful if additional examples could be found experimentally. It is likely that such studies of resonant transfer reactions will provide a useful opportunity to develop nuclear reaction theory.



Ratio of the cross section for the elastic scattering of alpha particles by ${}^7\text{Li}$ to the energy-averaged cross section as a function of energy showing oscillatory structure interpreted as due to a resonant transfer of a triton between the two α particles. The dashed curves show the variation expected from the simple theory of the resonant transfer process.

The Logic of Animal Conflict

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Conflicts between animals of the same species usually are of "limited war" type, not causing serious injury. This is often explained as due to group or species selection for behaviour benefiting the species rather than individuals. Game theory and computer simulation analyses show, however, that a "limited war" strategy benefits individual animals as well as the species.

In a typical combat between two male animals of the same species, the winner gains mates, dominance rights, desirable territory, or other advantages that will tend toward transmitting its genes to future generations at higher frequencies than the loser's genes. Consequently, one might expect that natural selection would develop maximally effective weapons and fighting styles for a "total war" strategy of battles between males to the death. But instead, intraspecific conflicts are usually of a "limited war" type, involving inefficient weapons or ritualized tactics that seldom cause serious injury to either contestant. For example, in many snake species the males fight each other by wrestling without using their fangs^{1,2}. In mule deer (*Odocoileus hemionus*) the bucks fight furiously but harmlessly by crashing or pushing antlers against antlers, while they refrain from attacking when an opponent turns away, exposing the unprotected side of its body³. And in the Arabian oryx (*Oryx leucoryx*) the extremely long, backward pointing horns are so inefficient for combat that in order for two males to fight they are forced to kneel down with their heads between their knees to direct their horns forward⁴. (For additional examples, see Collins⁵, Darwin⁶, Hingston⁶, Huxley *et al.*⁷, Lorenz⁸ and Wynne-Edwards⁹.)

How can one explain such oddities as snakes that wrestle with each other, deer that refuse to strike "foul blows", and antelope that kneel down to fight?

The accepted explanation for the conventional nature of contests is that if no conventional methods existed, many individuals would be injured, and this would militate against the survival of the species (see, for example, Huxley⁷). The difficulty with this type of explanation is that it appears to assume the operation of "group selection". Although one cannot rule out group selection as an agent producing adaptations, it is only likely to be effective in rather special circumstances¹⁰⁻¹². Consequently it seems to us that group selection cannot by itself account for the complex anatomical and behavioural adaptations for limited conflict found in so many species, but there must also be individual selection for these, which means that a "limited war" strategy must be differentially advantageous for individuals.

We consider simple formal models of conflict situations,

and ask what strategy will be favoured under individual selection. We first consider conflict in species possessing offensive weapons capable of inflicting serious injury on other members of the species. Then we consider conflict in species where serious injury is impossible, so that victory goes to the contestant who fights longest. For each model, we seek a strategy that will be stable under natural selection; that is, we seek an "evolutionarily stable strategy" or ESS. The concept of an ESS is fundamental to our argument; it has been derived in part from the theory of games, and in part from the work of MacArthur¹³ and of Hamilton¹⁴ on the evolution of the sex ratio. Roughly, an ESS is a strategy such that, if most of the members of a population adopt it, there is no "mutant" strategy that would give higher reproductive fitness.

A Computer Model

A main reason for using computer simulation was to test whether it is possible even in theory for individual selection to account for "limited war" behaviour.

We consider a species that possesses offensive weapons capable of inflicting serious injuries. We assume that there are two categories of conflict tactics: "conventional" tactics, *C*, which are unlikely to cause serious injury, and "dangerous" tactics, *D*, which are likely to injure the opponent seriously if they are employed for long. (Thus in the snake example, wrestling involves *C* tactics and use of fangs would be *D* tactics. In many species, *C* tactics are limited to threat displays at a distance, without any physical fighting. We consider a conflict between two individuals to consist of a series of alternate "moves". At each move, a contestant can employ *C* or *D* tactics, or retreat, *R*. If a contestant employs *D* tactics, there is a fixed probability that his opponent will be seriously injured: a contestant who is seriously injured always retreats. If a contestant retreats, the contest is at an end and his opponent is the winner. A possible conflict between contestants *A* and *B* can be represented in this way:

A's move C C C C C C C C C C C C D C C C C C C C D

B's move C C C C C C C C C C C C D C C C C C C C R

If a contestant plays *D* on the first move of a contest, or plays *D* in response to *C* by his opponent, this is called a "probe" or a "provocation". A probe made after the opening move is said to "escalate" a contest from *C* to *D* level. A contestant who plays *D* in reply to a probe is said to "retaliate". In the example shown above, *A* probes on his twelfth and twentieth moves; *B* retaliates after the first probe, but retreats after the second, leaving *A* the winner. At the end of a contest there are "pay-offs" to each contestant. The pay-offs are taken as measures of the contribution the contest has made to the reproductive success of the individual. They take account of three factors: the advantages of winning as compared with losing, the disadvantage of being seriously injured, and the disadvantage of wasting time and energy in the contest.

A "strategy" for a contestant is a set of rules which ascribe probabilities to the *C*, *D*, and *R* plays, as functions of what has previously happened in the course of the current contest. (No memory of what has happened in previous contests with the same or other opponents is assumed.) For computer simulation we programmed five possible strategies, each of which might be thought on *a priori* grounds to be optimal in certain circumstances. The strategies considered were as follows:

(1) "Mouse". Never plays *D*. If receives *D*, retreats at once before there is any possibility of receiving a serious injury. Otherwise plays *C* until the contest has lasted a preassigned number of moves.

(2) "Hawk". Always plays *D*. Continues the contest until he is seriously injured or his opponent retreats.

(3) "Bully". Plays *D* if making the first move. Plays *D* in response to *C*. Plays *C* in response to *D*. Retreats if opponent plays *D* a second time.

(4) "Retaliator". Plays *C* if making the first move. If opponent plays *C*, plays *C* (but plays *R* if contest has lasted a preassigned number of moves). If opponent plays *D*, with a high probability retaliates by playing *D*.

(5) "Prober-Retaliator". If making the first move, or after opponent has played *C*, with high probability plays *C* and with low probability plays *D* (but plays *R* if contest has lasted a preassigned number of moves). After giving a probe, reverts to *C* if opponent retaliates, but "takes advantage" by continuing to play *D* if opponent plays *C*. After receiving a probe, with high probability plays *D*.

The contestants were programmed as having identical fighting prowess, so that they differed only in the strategies they followed. The five strategies represent extremes, but from results with these it is possible to estimate the results likely to be found with intermediate types. The Hawk strategy is a "total war" strategy; Mouse, Retaliator, and Prober-Retaliator are "limited war" strategies. The question of main interest is whether individual selection will favour the former or one of the latter types.

The Simulation Test

The five strategies determine fifteen types of two-opponent contests. Two thousand contests of each type were simulated by computer, using pseudo-random numbers generated by an algorithm to vary the contests. The following probabilities were used: Probability of serious injury from a single *D* play = 0.10. Probability that a Prober-Retaliator will probe on the opening move or after opponent has played *C* = 0.05. Probability that Retaliator or Prober-Retaliator will retaliate against a probe (if not injured) by opponent = 1.0. Pay-offs were calculated as follows: Pay-off for winning = +60. Pay-off for receiving serious injury = -100. Pay-off for each *D* received that does not cause serious injury (a "scratch") = -2. Pay-off for saving time and energy (awarded to each contestant not seriously injured) varied from 0 for a contest of maximum length, to +20 for a very short contest. The contest example shown earlier was one of the 2,000 Prober-Retaliator versus Prober-Retaliator contests.

Table 1 shows the average pay-off to each contestant in each type of contest. The number in a given row and

column is the pay-off gained by the row strategy when the opponent uses the column strategy. For example, in contests between Mouse and Hawk, the average pay-offs are 19.5 to Mouse and 80.0 to Hawk.

To tell whether a strategy is evolutionarily stable against the other four strategies, we examine the corresponding column in Table 1. For example, for Hawk to be an ESS, it is necessary that it be the most profitable strategy in a population almost entirely of Hawks. In such a population, a given animal of any type will almost always have a Hawk as opponent. Therefore the pay-offs in the "Hawk" column apply. These show that Mouse and Bully are both more successful than Hawk. Therefore natural selection will cause alleles for Mouse and Bully behaviour to increase in frequency, and alleles giving Hawk behaviour to decrease. Thus Hawk is not an ESS.

Examining the other columns, we see that Mouse is not an ESS because Hawk, Bully, and Prober-Retaliator average higher pay-offs in a population almost entirely of Mouse. Nor is Bully an ESS. However, Retaliator is an ESS since no other strategy does better, though Mouse does equally well. And the last column shows that Prober-Retaliator is almost an ESS.

How would we expect such a population to evolve? It will come to consist mainly of Retaliators or Prober-Retaliators, with the other strategies maintained at a low frequency by mutation. The balance between the two main types will depend on the frequency of Mouse, since the habit of probing is only an advantage against Mouse. For the particular values in Table 1, it can be shown that if the frequency of Mouse is greater than 7%, Prober-Retaliator will replace Retaliator as the predominant type. It is worth noting that a real population would contain young, senile, diseased and injured individuals adopting the strategy Mouse for non-genetic reasons.

Thus the simulation shows emphatically the superiority, under individual selection, of "limited war" strategies in comparison with the Hawk strategy.

Briefly, the reason that conflict limitation increases individual fitness is that retaliation behaviour decreases the fitness of Hawks, while the existence of possible future mating opportunities reduces the loss from retreating uninjured.

This general result will not be altered by moderate changes in the program parameters, though very large changes will alter it. One way would be by changing the probability of serious injury from a single *D* from 0.10 to 0.90. This would give advantage to "Pre-emptive Strike" policies, making Hawk an ESS. (Such species are probably rare, because excessively dangerous weapons or tactics would be opposed by kin selection.) Another way to make selection favour "total war" behaviour would be by giving the same pay-off penalty for retreating uninjured as for serious injury. This would correspond to a species where an individual fights only a single battle in its lifetime, on which its reproductive success entirely depends. Our choice of +60 for winning, 0 for retreating uninjured, and -100 for serious injury represents a species where males have more than one opportunity to gain a mate. Changing these values to +60, -100, and -100 respectively, would make Hawk the optimal strategy. Conversely, +60, 0,

Table 1 Average Pay-offs in Simulated Intraspecific Contests for Five Different Strategies

		Opponent				
Contestant receiving the pay-off	"Mouse"	"Mouse"	"Hawk"	"Bully"	"Retaliator"	"Prober-Retaliator"
	"Mouse"	29.0	19.5	19.5	29.0	17.2
	"Hawk"	80.0	-19.5	74.6	-18.1	-18.9
	"Bully"	80.0	4.9	41.5	11.9	11.2
	"Retaliator"	29.0	-22.3	57.1	29.0	23.1
	"Prober-Retaliator"	56.7	-20.1	59.4	26.9	21.9

—500 would represent a long-lived species with numerous opportunities to gain mates, where individual selection would still more strongly favour cautious strategies.

Real Animals

Real animal conflicts are vastly more complex than our simulated conflicts. (An interesting study by Dingle¹⁵ shows that this holds true even at the lowly level of the mantis shrimp.) Probably our models are true to nature in emphasising a category distinction rather than an intensity distinction between “conventional” and “dangerous” tactics. In real animals, however, there exist not only the category distinction, but also individual differences in the intensity and skill with which each kind of tactic is employed. Also, in many species there are several categories of increasingly dangerous tactics, instead of only one.

The advantage from making a category distinction is that this simplifies behavioural requirements for limited conflict. It is probably easier for genetics to program a snake not to use fangs at all in certain situations than to program it to use fangs as intensively as possible up to intensity k , but not at intensities greater than k . Similarly, fair and foul blows are distinguished in boxing, and conventional and nuclear weapons in war.

Under the condition that any act of physical aggression is treated as a D act, the theoretical model will result in symbolic fighting by threat from a distance. This would be advantageous for a species that has an inherent difficulty in fighting physically at a safe level. For example, domestic and wild cattle, which have very dangerous horns and are somewhat clumsy in their charges, make much use of threat displays (stomping, pawing, bellowing). The model will not, however, give rise to conflict behaviour that is wholly symbolic and never backed up by physical aggression or other sanctions, since such behaviour would not be evolutionarily stable without some mechanism reducing the reproductive success of mutant individuals deficient in responding to the symbols. An interesting problem is how the felids, with their dangerous teeth and claws, limit their physical combats to non-fatal levels. Probably the explanation is that they have a hierarchy of many conflict categories and limit their probing to small escalations. Consequently, it takes repeated escalations to raise the conflict to the most dangerous level.

In most animal species there is probably a high correlation between prowess in C tactics and in D tactics. This means that C level conflict provides information to each animal about how its opponent is likely to perform if the conflict is escalated. This permits improvement in strategies over those used in the computer model. Instead of probing at random, an animal will be more likely to probe if its opponent is inferior in conventional fighting. On the other hand, if its opponent is very superior in conventional tactics, an animal will frequently retreat without waiting for its opponent to try a probe. Thus actual animals may combine Prober-Retaliator and Mouse capabilities.

If animals can adopt different strategies according to the opponent that confronts them, then an interesting possibility appears. The “Hawk” column of Table 1 shows that the best strategy against a Hawk is Mouse: that is, retreat immediately. If a species includes deviant individuals who follow the Hawk strategy and fight recklessly against every opponent, then it will be advantageous for ordinary members of the species to be able to estimate recklessness and avoid combat with Hawks. But if this happens, then it will be advantageous to simulate wild, uncontrollable rage. And in fact the threat displays of some species do have an appearance of maniacal fury, hence there probably is some advantage in acting this way. However, if most species members simulate insane rage when actually their fighting is limited and controlled, then selection will favour indi-

viduals who partly discount the threat displays, and “call the bluff” of the pseudo-Hawks.

This leads to the suggestion that it might be advantageous for an individual animal to be maniacal in an easily recognisable way that could not be counterfeited. A possible instance of this is the phenomenon of going “on musth”, which occurs periodically in adult male elephants^{16,17}. The temporal glands secrete a dark brown fluid that runs down the face, giving a visual and olfactory sign that cannot be counterfeited. The madness of the animal “on musth” causes other elephants to avoid him, and this may give an increase in dominance status that persists for a time after the musth period is over.

Conflict in which Injury is Impossible

The previous section offers an explanation of why, in a species with offensive weapons capable of inflicting serious injury, escalated fighting may be rare or absent. In doing so, it raises a second problem. In a contest between opponents who are unable to inflict serious injury, victory goes to the one who is prepared to continue for a longer time. How are such contests decided?

Suppose that the pay-off to the victor is v . If a contest is ever to be settled, there must also be some disadvantage to the contestants in a long contest. If so, the only choice of strategy open to a contestant is of the period for which he is prepared to continue, and hence of the pay-off, say $-m$, he is prepared to accept. Thus if two contestants adopt strategies m_1 and m_2 , where $m_1 > m_2$, the pay-off to the first is $v - m_2$ and to the second is $-m_2$. Our problem then is how a contestant should choose a value of m , or, more precisely, whether there is a method of choosing m which is an ESS.

To answer this question, we need a more precise definition of an ESS. We define $E_J(I)$ as the expected pay-off to I played against J . Then I is an ESS if, for all J , $E_I(I) > E_I(J)$; if for any strategy J , $E_I(I) = E_I(J)$, then evolutionary stability requires that $E_J(I) > E_J(J)$. The relevance of the latter condition is as follows. If in a population adopting strategy I a mutant J arises whose expectation against I is the same as I 's expectation against itself, then J will increase by genetic drift until meetings between two J 's becomes a common event.

It is easy to show that no “pure” strategy (that is, no fixed value of m) is an ESS. Thus in a population adopting strategy m , a mutant adopting $m + \epsilon$ would always do better (and if $m > v$, a mutant adopting a zero strategy would also do better). It is, however, possible to find a mixed strategy which is an ESS. Let strategy I be a mixed strategy which selects a value of m between x and $x + \delta x$ with probability $p(x)\delta x$.

Then if

$$p(x) = (1/v)\exp(-x/v) \quad (1)$$

it can be shown that I is an ESS.

We conclude that an evolutionary stable population is either genetically polymorphic, the strategies of individuals being distributed as in equation (1), or that it consists of individuals whose behaviour differs from contest to contest as in (1). There is no stable pure strategy, and hence no behaviourally uniform population can be stable.

Conclusions

There are many complications left out of these simple models. The analysis is, however, sufficient to show that individual selection can explain why potentially dangerous offensive weapons are rarely used in intraspecific contests; a stable strategy does, however, require that contestants should respond to an “escalated” attack by escalating in return. Also, if contests are settled by a process of attrition, then evolutionary stability requires that the popula-

tion be genetically polymorphic, or that individuals vary their behaviour from contest to contest.

A more detailed analysis will be published elsewhere.

Ideas similar to those described here have been applied to human neurotic behaviour by J. S. Price¹⁸.

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¹ Shaw, C. E., *Herpetologica*, **4**, 137 (1948).

² Shaw, C. E., *Herpetologica*, **7**, 149 (1951).

³ Linsdale, J. M., and Tomich, P. Q., *A Herd of Mule Deer*, 511f (Univ. of California Press, Berkeley and Los Angeles, 1953).

⁴ Darwin, C., *The Descent of Man and Selection in Relation to Sex*, chap. 17 (Murray, London, 1882).

⁵ Collins, N. E., *Physiol. Zool.*, **17**, 83 (1944).

⁶ Hingston, R. W. G., *Character Person.*, **2**, 3 (1933).

⁷ Huxley, J. S., *Phil. Trans. R. Soc.*, **251B**, 249 (1956).

⁸ Lorenz, K., *On Aggression* (Methuen, London, 1966).

⁹ Wynne-Edwards, V. C., *Animal Dispersion in Relation to Social Behaviour*, chap. 8 (Oliver and Boyd, Edinburgh and London, 1962).

¹⁰ Maynard Smith, J., *Nature*, **201**, 1145 (1964).

¹¹ Levins, R., in *Some Mathematical Questions in Biology* (American Mathematical Society, 1970).

¹² Price, G. R., *Ann. hum. Genet.*, **35**, 485 (1972).

¹³ MacArthur, R. H., in *Theoretical and Mathematical Biology* (edit. by Waterman, T., and Horowitz, H.) (Blaisdell, New York, 1965).

¹⁴ Hamilton, W. D., *Science, N.Y.*, **156**, 477 (1967).

¹⁵ Dingle, H., *Anim. Behav.*, **17**, 561 (1969).

¹⁶ West, L. J., Pierce, C. M., and Thomas, W. D., *Science, N.Y.*, **138**, 1100 (1962).

¹⁷ Eisenberg, J. F., McKay, G. M., and Jainudeen, M. R., *Behaviour*, **38**, 193 (1971).

¹⁸ Price, J. S., *Proc. R. Soc. Med.*, **62**, 1107 (1969).

Lithospheric Plate Motion, Sea Level Changes and Climatic and Ecological Consequences

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We demonstrate quantitatively that the world-wide Mid to Upper Cretaceous transgression and subsequent regression may have been caused by a contemporaneous pulse of rapid spreading at most of the mid-oceanic ridges between -110 to -85 m.y. The rapid spreading caused the ridges to expand and hence reduced the volumetric capacity of the basins. The subsequent regression was caused by a reduction in spreading rates beginning at -85 m.y.

THE global nature of the great marine transgressions and regressions such as occurred in the Upper Cretaceous has been recognised for nearly a century¹. These fluctuations of sea level have been variously attributed to gradual filling of ocean basins by detritus displacing water onto the continents, down faulting of ocean basins to cause regressions¹ and simultaneous vertical movements of both continents and ocean basins^{2,3}. The transgressions and regressions have been linked to orogenic cycles; it has been argued² that during intervals of low orogenic activity reduced horizontal compression caused continental subsidence relative to sea level, hence transgressions, and that conversely orogenic episodes produced increased horizontal compression, increased continental elevation and marine regression⁴. Late Cainozoic sea level changes are certainly attributable to glaciation, but this cause is not applicable to the Upper Mesozoic.

Changes in the volume of ocean basins could explain flooding of portions of continental surfaces. It has been

proposed⁵⁻⁷ that such changes occur due to alterations in the volume of the mid-oceanic ridges. Valentine and Moores⁵ linked these volumetric changes to the assembly and breakup of super continents. Hallam⁶ speculated that the Upper Cretaceous transgression and regression may have been caused by a contemporaneous pulse of rapid spreading which substantially increased the volume of the world ridge system. We show that this latter proposal is correct.

Cause of Upper Cretaceous Transgression

The lithosphere formed at a spreading ridge axis is initially hot and therefore elevated; as it moves away from the axis, it cools and subsides^{8,10}. This cooling and subsidence is time dependent¹¹; the depth to which any portion of flanking crust has subsided is essentially a function of age only. So one empirical age-depth relation fits most ridges regardless of spreading rate¹¹ (Table 1). Therefore, the volume of any ridge is a function of its spreading rate history and changes in the spreading rate cause, in time, changes in ridge volume. (It has been suggested that the axial portion of fast spreading ridges is deeper than that of slow spreading ridges¹¹; however, subsequent analysis has shown that this is not systematically true¹².) Larson and Pitman¹³ correlated anomaly lineations of Middle and Upper Mesozoic age in the Atlantic and Pacific. They calibrated these lineations with Deep-Sea Drilling Project data, thereby extending the magnetic polarity time scale to -160 m.y. From the geometry of these lineations they showed that during the Upper Cretaceous (-110 m.y. to -85 m.y.) there was an episode of rapid spreading in the central and south Atlantic and the Pacific. The geological evidence suggests an initial rise of sea level that began at or before the boundary between Upper and Lower Cretaceous (-100 m.y.), the cresting of this rise some time between the Turonian and Lower Maastrichtian (-90 and -70 m.y.) and a withdrawal that was most pronounced in the Maastrichtian but continued into the Cainozoic¹⁴⁻¹⁷.

Table 1 Empirical Values showing the Relationship between Crustal Age and Ridge Elevation¹¹

Age m.y.	Mean depth corrected (m)	Standard deviation (m)	No. of values	Mean depth at 5 m.y. intervals (m)	Elevation above 5.5 base level (m)
0	2,644	±109	40	2,644	2,856
2	2,948	±93	46		
4	3,178	±96	46		
5				3,243	2,257
6	3,309	±94	33		
8	3,480	±81	32		
10	3,545	±155	51	3,545	1,955
15				3,788	1,712
20				4,029	1,471
21	4,078	±33	33		
25				4,205	1,295
29	4,332	±17	17		
30				4,371	1,129
33.5	4,507	±77	13		
35				4,556	944
38	4,655	±46	27		
40				4,718	782
45				4,879	621
50				5,041	459
53	5,137	±47	20		
64	5,432	±100	15		
69.3				5,500	0
77	5,600	±63	13		

We use the Upper Cretaceous system of ridges proposed by Larson and Pitman¹³ (Fig. 2a, Table 3). In the Atlantic this system has not altered significantly since -180 m.y. except to extend both northwards and southwards. The Pacific ridges, however, are today different from the Cretaceous configuration¹³ (Fig. 2, a and c). For example, the lineations of Mesozoic age form a double magnetic bight indicating that a ridge-ridge-ridge triple junction must have existed at that time at points A and B of Fig. 2a. This system of ridges evolved into the present system (Fig. 2c). The Phoenix plate is now the Antarctic plate and Juan de Fuca, Cocos and Nasca plates are all that remain of the Farallon plate; the Kula plate is gone. The Galapagos Ridge (between the Cocos and Nasca plates) is no more than 10 m.y. old and does not affect our computations. Beginning at -75 m.y. there was a phase of spreading at about 3 cm yr⁻¹ between the small remaining eastern fragment of the Kula plate and the Pacific plate. (This event yielded the "Great Magnetic Bight of the North-east Pacific"¹⁸.) By -38 m.y. at the latest this spreading had ceased and the entire Kula plate with its bordering ridges had been subducted¹⁹. The volumetric increase due to this brief episode would have been small.

In calculating the volumetric changes for each interval, we have kept the length of each ridge segment fixed. In reality, the length of the ridges changed with time, but

Table 2 The Calculated Volume of the Ridge Segments at Various Times is Given as are the Changes in Total Volume and Sea Level

	-110	-100	-90	-85	-80	-70	-60	-50	-40	-30	-20	-10	
PAC-PH	24.2	44.3	59.0	64.9	57.5	47.5	39.1	32.6	26.3	22.0	20.0	19.4	Volume × 10 ⁶ (km ³)
PH-FAR	21.8	35.7	45.9	50.0	42.9	33.0	24.9	18.6	13.1	9.4	7.6	7.3	
PAC-FAR	26.7	43.7	56.1	61.1	57.7	53.7	48.2	44.6	39.1	37.1	35.8	35.6	
KUL-FAR	36.4	59.5	76.4	83.3	78.7	71.9	65.8	60.9	53.3	50.6	48.8	48.5	
PAC-KUL	51.8	49.4	44.7	43.4	35.6	23.6	14.6	7.9	3.8	1.3	0.1	0	
A-NA	6.5	10.6	13.6	14.8	14.8	14.7	14.5	14.3	13.6	13.2	13.0	12.9	
A-SA	0.0	11.5	20.0	23.4	22.6	21.5	20.1	19.3	16.7	15.3	14.6	14.6	
E-NA					2.0	5.0	7.3	9.0	10.2	10.9	11.2	11.3	
ΔV × 10 ⁶ km ³		85.4	148.2	173.7	144.9	102.8	67.5	40.0	9.7	-5.6	-14.3	-15.7	
h-d m		212	362	421	354	254	168	100	24.0	-14.0	-33.0	-40.0	

Data and Computations

We have computed the volume of most of the mid-oceanic ridge system at several times, beginning in the Cretaceous and extending to the upper Tertiary (Table 2). For each time and for each ridge segment an average cross-sectional area was computed using a standard age-depth curve¹¹ (Table 1). The data are referred to present day sea surface. Since sea level varies with time it is more meaningful to consider ridge volume in terms of elevation above an abyssal reference plain of 5,500 m. A history of spreading rates for the ridge segments is adapted from Larson and Pitman¹³ (Table 3).

Table 3 Lengths and Half Spreading Rates of Ridge Segments from -110 m.y. to -9 m.y.

	Length of ridge (km)	Half spreading rates (cm yr ⁻¹)		
		Prior to 110 m.y.	110-85 m.y.	85-10 m.y.
Pacific-Phoenix (PAC-PH)	3,300	5	18	4
Phoenix-Farallon (PH-FAR)	4,950	3	9	1
Pacific-Farallon (PAC-FAR)	6,050	3	9	4
Kula-Farallon (KUL-FAR)	8,250	3	9	4
Pacific-Kula (PAC-KUL)	8,800	4	3	0
Africa-North America (A-NA)	4,400	1	3	2
Africa-South America (A-SA)	4,950	0	5	2
Europe-North America (E-NA)	3,850	0	0	2
Total	45,550			

we assume that as some ridges have grown in length an equivalent volume of other ridges has been subducted.

The Indian Ocean and Tethys Sea have been left out of our calculation. The spreading of the Indian Ocean is known in sufficient detail¹⁰ but since the Tethys is gone its history cannot be determined. As a first approximation we assume that as Tethys closed due to the drift of various Gondwana fragments into Eurasia, any ridge system that existed in Tethys gradually subsided, ridges and ridge axes were subducted. We also assume that this consequent volumetric decrease was about equal to the simultaneous volumetric increase in the ridges of the Indian Ocean.

To convert changes of ridge volume into changes of continental freeboard, we calculated isostatic adjustment due to increased water depth. If water depth increases by a thickness h , oceans subside a distance d . Assuming 3.3 g cm⁻³ for the density of the upper part of the mantle, $h=3.3d$ and the change in freeboard will be $(h-d)=0.7h$.

A second correction arises because the boundaries of the oceans are not vertical. As the oceans rise, more surface area is covered (Fig. 1). Approximately 1/6 of the Earth's surface (85×10^6 km²) lies at elevations between 0 and +500 m (ref. 20). We assume that as sea level rises the area covered by the sea increases linearly (0.170×10^6 km² for each 0.001 km rise in sea level). Thus, the change in continental freeboard $(h-d)$ may be calculated from the equation

$$V = hA_0 + (0.7h)^2 170/2$$

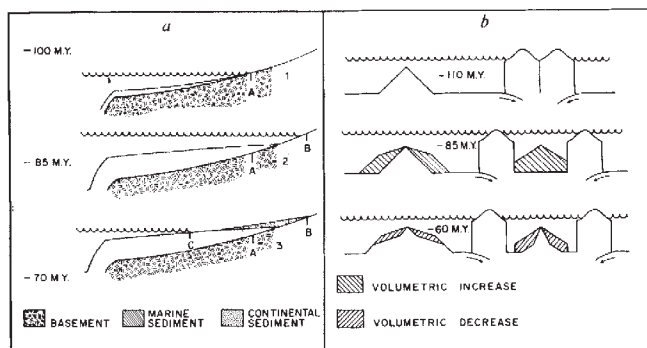


Fig. 1 *a*, Schematic Upper Cretaceous two ocean ridge system. As spreading rates increase at -110 m.y. ridges expand horizontally causing a transgression by -85 m.y. Decrease in spreading rates after -85 m.y. causes ridges to contract and the seas to withdraw. *b*, As sea level rises from -100 m.y. (A) to -85 m.y. (B), sediments fill and level the inland seafloors. In contrast, as sea level lowers an equivalent amount between -85 and -70 m.y., the area of continent exposed is much greater (curve B to C).

where A_0 is the surface area of the ocean at -110 m.y. and $(0.7h)^2/170/2$ is the volume occupied by the sea encroaching on the continent. Hallam⁸, using the data of Termier and Termier²¹, shows that 33×10^6 km² of presently emergent crust was covered by seas at -110 m.y. The present area occupied by the oceans is 359×10^6 km²; thus the oceanic area at -110 m.y. was 392×10^6 km². The freeboard change ($h-d$) has been calculated from the equation for each cumulative total change in volume.

The values of ($h-d$) given in Table 2 have been computed relative to sea level taken as zero at -110 m.y. But since 33×10^6 km² of the present day land area was covered by the seas⁸ at that time we estimate (using a standard hypometric curve²⁰) that sea level was about 100 m higher than today. The curve in Fig. 3 has been adjusted to give sea levels relative to the present.

Estimates of the late Mesozoic-Cainozoic changes in sea level computed from our model agree well with other estimates based on outcrops of marine sediments (Fig. 3). All except Termier and Termier²¹ place the time of the maximum transgression at about -85 m.y. Our curve indicates that sea level 10 m.y. ago was 40 m above the present stand. The total present continental ice volume, most of which accumulated during the past 10 to 20 m.y., is equivalent to 60 m of sea level change²³. Therefore, our model predicts a present day sea level within 20 m of that observed.

Our estimates differ from those inferred geologically by others²¹⁻²³ in two respects (Fig. 3). First, we obtain higher sea levels during the maximum transgression. Our calculation may contain errors inherent in our assumptions but the corresponding geological estimates of past marine transgressions are based on the extent of Upper Cretaceous marine sediments, and hence are low, since 70 m.y. of erosion must have removed some of the Upper Cretaceous deposits⁷. Second, our model predicts that the rise of sea level during the transgression will occur at about the same rate as the fall during the regression.

Some geological data point to a more rapid regression than transgression. But it is likely that sedimentation led to the continuous filling and levelling of the epicontinental seafloors. Therefore, depths of the Cretaceous epicontinental seas in general would have been less than 500 m, perhaps only 100 to 200 m as suggested by the limited palaeontological evidence²⁴. Consequently, the area drained by the first 200 m drop of sea level would have been very large relative to the area flooded by the latest 200 m rise of the transgression (Fig. 1*b*). We estimate that between -85 m.y. and -60

m.y. sea level would have fallen by more than 250 m. This would have been sufficient to drain most continental areas and is in agreement with geological evidence.

Consequences of our Model

What is the relationship between tectonism, climate change and fluctuations in faunal diversity? We believe that our

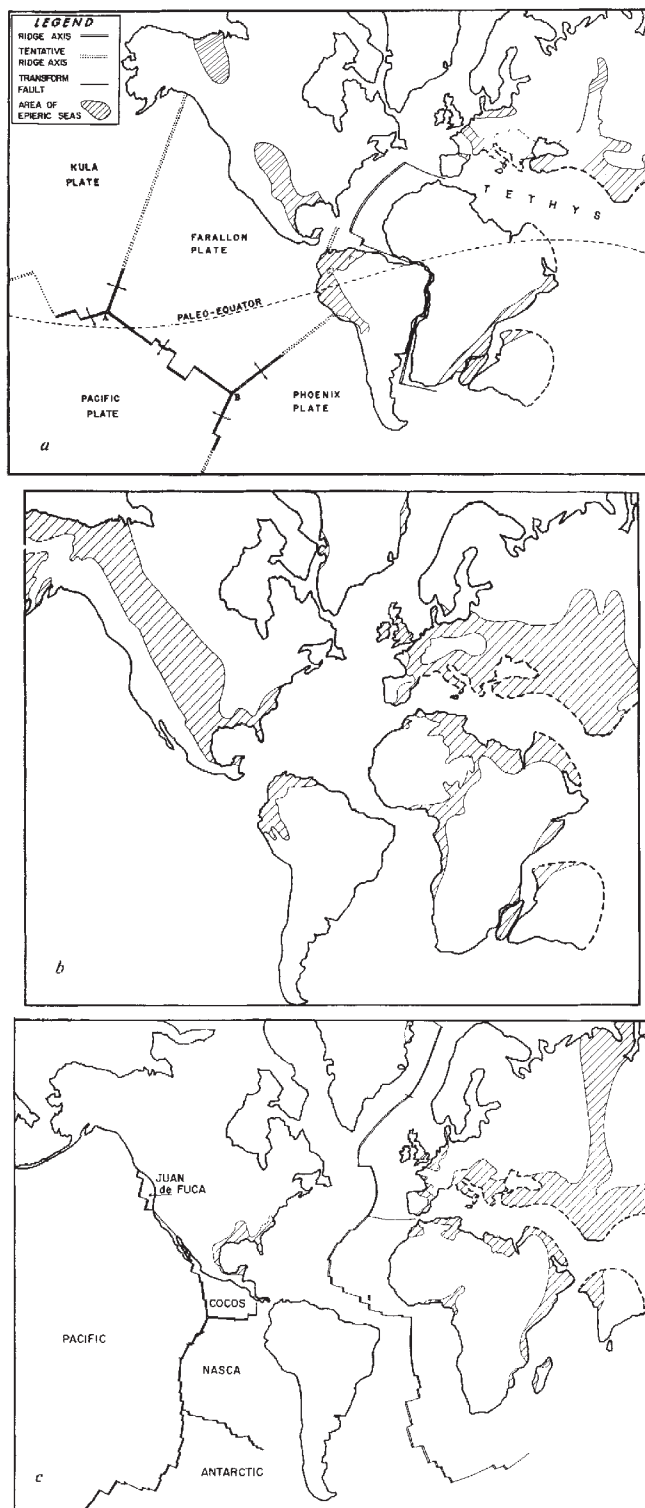


Fig. 2 The extent of the seas for three geological epochs^{14,16,17,21}: *a*, Cenomanian (-100 to -94 m.y.); *b*, Santonian-Campanian (-70 to -85 m.y.); *c*, Eocene (-50 to -40 m.y.). The arrangement of the ridge axes at present is shown in *c*, where they are plotted with respect to North America.

model provides a working hypothesis to connect these phenomena. The effects of the Upper Cretaceous transgression and regression on climatic, stability, faunal diversity and ocean circulation (Fig. 4) are not necessarily newly discovered relationships. But we discuss them briefly to emphasize that all are related to changes in the rate of plate motion.

World climate: The uniformity and relative mildness of climate at the height of the Upper Cretaceous transgression are well documented²⁵⁻²⁷. An increase in the ratio of oceanic to continental area moderates and stabilizes climate¹. This is because the heat capacity of water is very large compared with continental rocks or the atmosphere. With more than 40% of the continents covered by several hundred metres of water, this moderating effect must have been large. Also, the expanse of the epicontinental seas provided new corridors along which heat could be advectively transferred between low and high latitudes. Conversely, as the seas withdrew beginning in the Upper Cretaceous, continentality increased probably producing the well documented high latitude cooling and increased seasonal contrasts that continued through the Tertiary. This cooling trend leads to polar glaciation in the mid-Tertiary²⁸ and mid-latitude glaciation in the Quaternary. Palaeomagnetic data³⁶ demonstrate that the Arctic Ocean and possibly Antarctica had drifted to a polar position by the Upper Cretaceous^{30,36}. We therefore attribute these Late Cretaceous glaciations to continued fall of sea level (which caused increasing emergence of all continents, including Antarctica) and increased constriction of oceanic thermal advection between low latitudes and the Arctic Ocean. Post-Cretaceous opening of the North Atlantic did not significantly improve circulation with the Arctic because, as the Atlantic opened, routes to the Pacific closed³¹.

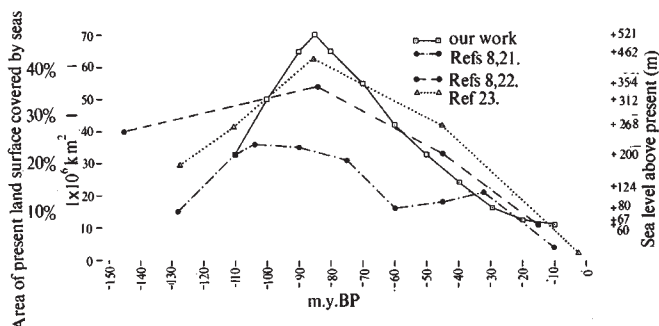


Fig. 3 Area of present day land covered by former seas plus the height of these seas above present levels plotted against age (adapted from ref. 8).

Ocean circulation: All available evidence suggests that the Upper Cretaceous oceans and their epeiric seas were well mixed and productive in spite of the low thermal gradient between poles and equator. The pattern of circulation is not known, but it was probably quite different from that of the present.

Today the deep circulation of the oceans is maintained by high latitude formation of dense water in restricted seas or shallow shelf areas. We expect that a similar mechanism operated in the Upper Cretaceous and the source areas for deep and bottom waters lay in some of the epeiric seas. With the withdrawal of the seas at the close of the Cretaceous some or all of these source areas may have been drained. New source areas would develop as the polar regions cooled but there may have been a short but important interval of stagnation as one regime of deep circulation was replaced by another. If a brief period of stagnation did occur there is no evidence that it led to the development of anaerobic conditions in any of the ocean basins.

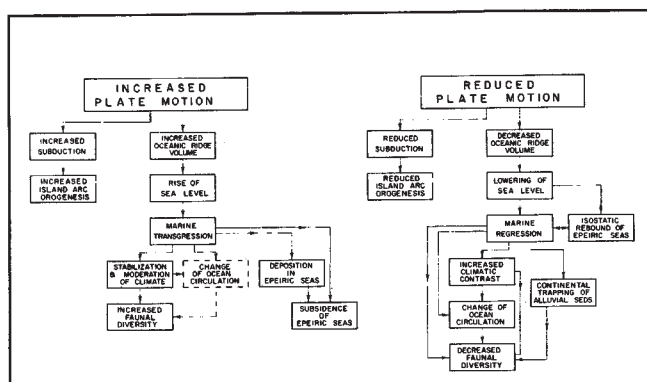


Fig. 4 Proposed sequence of events causally related to an Upper Cretaceous worldwide increase in the velocity of plate motion and subsequent decrease.

Mesozoic-Cainozoic faunal crisis: The Upper Cretaceous stable and uniform climates allowed significant increases in diversity of many groups^{32,33}. The adaptation to these conditions produced an inherent vulnerability to change³⁴. We conclude that the marine withdrawal was most rapid between -70 m.y. and -60 m.y. because of isostatic rebound and sediment infilling of epeiric seas (Fig. 1). Thus, in latest Cretaceous, the land area would have nearly doubled. The consequent environmental changes (increased thermal gradients and seasonal contrast^{33,35} and marked alteration of the ocean circulation and thermal regime) were probably equally rapid, producing stresses on species adapted to stable Upper Cretaceous conditions.

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1. Seuss, E., in *The Face of the Earth II* (Oxford University Press, 1906).
2. Stille, H., *Einführung in den Bau Amerikas* (Oebruder Borntraeger, Berlin, 1940).
3. Umgrove, J. H. F., in *The Pulse of the Earth*, second ed. (Nijhoff, The Hague, 1949).
4. Brooks, C. E. P., in *Climate Through the Ages* (Ernest Benn, London, 1949).
5. Valentine, J. W., and Moores, E. M., *Nature*, **228**, 657 (1970).
6. Menard, H. W., in *Marine Geology of the Pacific* (McGraw-Hill, New York, 1964).
7. Hallam, A., *Am. J. Sci.*, **201**, 397 (1963).
8. Hallam, A., *Nature*, **232**, 180 (1971).
9. Menard, H. W., *Earth planet. Sci. Lett.*, **6**, 275 (1969).
10. McKenzie, D., and Sclater, J. G., *Geophys. J. R. astr. Soc.*, **25**, 437 (1971).
11. Sclater, J., Anderson, R., and Bell, M. L., *J. geophys. Res.*, **32**, 7888 (1971).
12. Anderson, R. N., McKenzie, D. P., and Sclater, J. G., *Earth planet. Sci. Lett.*, **18**, 391 (1973).
13. Larson, R. L., and Pitman, W. C., III, *Bull. geol. Soc. Am.*, **83**, 3645 (1972).
14. Gignoux, M., *Stratigraphic Geology: Translation of fourth French Edition 1950*, 1-16 (Freeman, San Francisco, 1955).
15. Naidin, D. P., in *Stockholm Contrib. Geol.* (edit. by Gavelin, S., and Hesseland, I.), **3**, 127 (1960).
16. Schuckert, C., in *Atlas of Paleogeographic Maps of North America* (Wiley, New York, 1955).
17. Brinkman, R., *The Geological Evolution of Europe* (Hafner, New York, 1960).
18. Elvers, D. J., Mathewson, C. C., Kohler, R. E., and Moses, R. L., in *Operational Data Report C and GSDR-1* (1967).
19. Atwater, T., *Bull. geol. Soc. Am.*, **81**, 351 (1970).
20. Sverdrup, H. U., Johnson, M. W., and Fleming, R. H., in *The Oceans* (Prentice Hall, Englewood Cliffs, New Jersey, 1942).
21. Termier, H., and Termier, G., in *Atlas de Paleogeographie* (Masson, Paris, 1960).
22. Strakhov, N. M., *Stages in the development of external geospheres and the formation of deposits in the history of the Earth* (Akad. Nauk, SSSR, Geol. Ser., **12**: 3-22).
23. Ronoff, A. B., *Sedimentology*, **10**, 25 (1968).
24. Ollson, R. K., *Bull. Am. Ass. Petrol. Geol.*, **47**, 654 (1963).

- ²⁵ Lowenstam, H. A., and Epstein, S., *Proc. Int. geol. Congr. twentieth Symp.*, 1, 65 (1959).
- ²⁶ Dorf, E., *Am. Scient.*, 48, 364 (1960).
- ²⁷ Newell, N., *Geol. Soc. Am. Spec. Pap.*, 89, 63 (1967).
- ²⁸ Denton, G., Armstrong, R. L., and Stuiver, M., in *Late Cenozoic Glacial Ages* (edit. by Turekian, K. K.) (Yale University Press, 1971).
- ²⁹ Donn, W. L., and Ewing, M., *Science, N.Y.*, 152, 3730 (1966).
- ³⁰ Pitman, W. C., III, and Talwani, M., *Bull. geol. Soc. Am.*, 83, 619 (1972).
- ³¹ Talwani, M., and Edholm, O., *Bull. geol. Soc. Am.*, 83, 3575 (1972).
- ³² Tappan, H., *Palaeogeogr., Palaeoclim., Palaeoecol.*, 4, 187 (1968).
- ³³ Newell, N., *Am. Mus. Novitates*, 2465 (1971).
- ³⁴ Bretsky, P. W., and Lorenz, D. M., *Bull. geol. Soc. Am.*, 81, 2449 (1970).
- ³⁵ Axelrod, D. F., and Bailey, H. P., *Evolution*, 22, 595 (1968).
- ³⁶ McElhinny, M. W., *Paleomagnetism and Plate Tectonics* (Cambridge University Press, 1973).

Possibilities for the Evolution of the Genetic Code from a Preceding Form

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Analysis of the interaction between mRNA codons and tRNA anticodons suggests a model for the evolution of the genetic code. Modification of the nucleic acid following the anticodon is at present essential in both eukaryotes and prokaryotes to ensure fidelity of translation of codons starting with A, and the amino acids which could be coded for before the evolution of the modifying enzymes can be deduced.

THE sequences of nucleotide bases in genetic DNA and in the corresponding mRNA molecules store inherited information which is translated into polypeptide chains of proteins by an adapter mechanism. This involves pairing between anticodons in tRNA molecules, carrying specific amino acids, and codons in mRNA. The rules of pairing between tRNA and mRNA molecules result in the genetic code (Table 1). Alterations in the nucleotide sequences of tRNA molecules produce functional changes. Two of the functions of tRNA that can undergo such changes are codon-anticodon pairing, the site for which is located in the anticodon loop, and amino acid recognition, which seems to be determined by various components of the tRNA molecule, perhaps including its tertiary structure. In some tRNAs the amino acid acceptor stem is involved in synthetase recognition¹, but this rule does not hold generally, and Loftfield¹ has concluded that amino acid recognition appears to be a property of the tRNA molecule as a whole, and cannot be ascribed to any particular sequence of bases in it.

The anticodon is a sequence of three nucleotides next in sequence to an invariant uridine in the anticodon loop. Mutational changes in the anticodon can take place that change codon pairing without affecting amino acid recognition². Such mutations result in the 'wrong' amino acid being placed in many polypeptide sequences, and may therefore be expected to be deleterious or lethal. tRNA molecules can also undergo changes in both the anticodon and the amino acid recognition function and such 'double

changes' can occur during evolution. This was discovered by Squires and Carbon³ who found that a glycine tRNA in *Escherichia coli* was similar to a valine tRNA isolated from the same organism with, of course, a different anticodon and different amino acid recognition function. Similarity, in excess of the similarity between all tRNA molecules for different amino acids, was also noted for lysine and arginine tRNAs occurring in yeast⁴.

Anticodon-Codon Pairing

In this article I develop the thesis that part of the evolution of the genetic code is brought to light by examining the nature of anticodon-codon pairing and by examining the four nucleotides that comprise the anticodon in tRNAs that participate in polypeptide synthesis on ribosomes and the nucleotide next following. This is always a purine nucleotide or its modification.

Ambiguity in the pairing between the first base of anticodons and the third base of codons (termed here, for convenience, '1-3 pairing') is responsible for a definite pattern in the genetic code⁵. The ordered ambiguity in 1-3 pairing was elegantly interpreted by Crick⁶, as shown in Table 2, in the cases of I, G, U and C in position 1 of anticodons. He proposed that these bases pair with three, two, two and one bases respectively in codons. Similarly, the base G in the third codon position will pair with both U and C in position 1 of the anticodon. Ambiguous pairing between position 2 of anticodon and codon does not exist and, with one exception to be discussed below, does not exist between anticodon position 3 and codon position 1 (3-1 pairing). Non-ambiguous 3-1 pairing takes place between A and U, and G and C. This may be attributed to conventional hydrogen bonding. Pairing between G and U is, however, known to take place in various RNA sequences without hydrogen bonding, and without displacing the helical structure of double strands of RNA⁶. This concept is basic to the understanding of 1-3 pairing. The ambiguity in base pairing between G with U and C, and U with A and G, potentially enables thirty-two anticodons to suffice for translation of all the codons for amino acids, for each pair of codons represented by NNU and NNC can bind with an anticodon GNN, 'read' in the reverse direction, and each NNA and NNG codon pair can bind with anticodon UNN (Table 1). In addition, there are anticodons that provide for duplicate translation of codons; for example, the anti-

Table 1 The Genetic Code and the Identified Anticodons in Sequenced tRNAs

<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>	
GAA	UUU	Phenylalanine	IGA	UCU	Serine	GUA	UAU	Tyrosine		UGU	Cysteine
UAA	UUC	Phenylalanine		UCC	Serine		UAC	Tyrosine		UGC	Cysteine
CAA	UUA	Leucine	UGA	UCA	Serine	UUA	UAA	Chain Term.		UGA	Chain Term.
	UUG	Leucine		UCG	Serine	CUA	UAG	Chain Term.	CCA	UGG	Tryptophan
GAG	CUU	Leucine		CCU	Proline	GUG	CAU	Histidine	ICG	CGU	Arginine
	CUC	Leucine		CCC	Proline		CAC	Histidine	GCG	CGC	Arginine
	CUA	Leucine		CCA	Proline	UUG	CAA	Glutamine		CGA	Arginine
CAG	CUG	Leucine		CCG	Proline	CUG	CAG	Glutamine		CGG	Arginine
IAU	AUU	Isoleucine		ACU	Threonine		AAU	Asparagine	GCU	AGU	Serine
GAU	AUC	Isoleucine		ACC	Threonine		AAC	Asparagine		AGC	Serine
	AUA	Isoleucine		ACA	Threonine	UUU	AAA	Lysine	UCU	AGA	Arginine
CAU	AUG	Methionine		ACG	Threonine	CUU	AAG	Lysine		AGG	Arginine
IAC	GUU	Valine	IGC	GCU	Alanine	GUC	GAU	Aspartic acid	GCC	GGU	Glycine
GAC	GUC	Valine		GCC	Alanine		GAC	Aspartic acid		GGC	Glycine
UAC	GUA	Valine		GCA	Alanine	UUC	GAA	Glutamic acid	UCC	GGA	Glycine
	GUG	Valine		GCG	Alanine		GAG	Glutamic acid	CCC	GGG	Glycine

a=Identified anticodons; *b*=codons.

The bases in anticodons are shown in unmodified form, except for I (inosine) which is a modification of A. The modifications are listed in Table 2.

Anticodons starting with G pair with codons ending in U and C; those starting with I pair with codons ending in U, C and A, and so on, as shown in Table 2.

codons UCC and CCC in glycine tRNAs both bind with the GGG codon for glycine.

Recent research by Nishimura and others (reviewed in ref. 7) has extended the pattern of ambiguity in 1-3 pairing. Uridine 5-oxyacetic acid in the first position of anticodons in certain *E. coli* tRNAs pairs with either U, A or G in the third codon position, and 2-thiouridine pairs only with A and not with G (ref. 7). Such modifications thus can either increase or decrease the ambiguity in 1-3 pairing. These enzymatic modifications of base 1 in anticodons vary in different organisms, and so have originated during the evolution of such organisms.

The presence of the invariant U preceding the anticodon would impose the minimum amount of steric hindrance to 1-3 pairing. This presumably facilitates pairing between modified bases at anticodon position 1 and codon position 3. Below, we shall contrast this with the effect of the modifications of the nucleoside following position 3 of the anticodon.

Table 2 First Base of Anticodons in Sequenced tRNAs compared with Third Base of Codons Paired

First base in anticodon	Pairing with	Organisms (E=eukaryotes P=prokaryotes)	In tRNAs for
H (I)	U, C, A	E, P	Ile, Val, Ser (UCN), Ala, Arg (CGN)
G	C, U	E, P	Phe, Leu (CUN), Ile, Val, Tyr, Asp, Arg (CGN), Gly, Ser (AGY)
Gm	C, U	E	Phe
G ⁺ ('Q')	C, U	P	Tyr, His, Asn, Asp
U	A, G	P	Gly, Term.
s ² U	A	E, P	Gln, Lys, Glu
oa ⁵ U	U, A, G	P	Val, Ser (UCN)
U ⁺	A, G (?)	E, P	Leu (UUR), Arg (AGR)
C	G	E, P	Leu (CUN, UUR), fMet, Gln, Lys, Trp, Gly, iMet
ac ⁴ C	G	P	Met
Cm	G	E	Trp

Abbreviations: H, hypoxanthine; I, inosine; oa⁵U, uridine-5-oxyacetic acid; Gm, 2'-O-methylguanosine; G⁺, 'Q-base' (an unidentified guanosine derivative); s²U, 2-thiouridine; U⁺, unidentified uridine derivative; ac⁴C, 4-acetylcytidine; Cm, 2'-O'-methylcytidine; N, unidentified nucleoside; R, purine; Y, pyrimidine; fMet, N-formyl methionine; iMet=initiator methionine.

Adenine is absent in position 1 of anticodons. Adenine in this position is deaminated to hypoxanthine by a specific enzyme, adenine anticodon deaminase (ACD)⁸. Hypoxanthine, which is the base in the nucleoside inosine (I), pairs with U, C, and A (Table 2). Anticodons with I in position 1 can therefore exist for amino acids that have quartets of four codons that start with the same two bases (see, for example, the set of alanine codons in Table 1), but not for amino acids with two codons in a set (such as phenylalanine), because in the latter case, anticodons starting with I would transfer two different amino acids to the same codon. Anticodons starting with A, which should translate codons ending in U, therefore do not exist in living systems, and mutations that bring such anticodons into being in tRNAs for amino acids having two codons in a set are evidently discarded because the enzyme ACD presumably changes A in this position to I in any and all tRNA molecules. All tRNAs that have been sequenced which translate pairs of codons ending in pyrimidines have anticodons starting with G, when such pairs of codons are assigned to a single amino acid, provided that the corresponding pair of codons ending in purines is assigned to a different amino acid. This has been found so far for the tRNAs for Phe, Tyr, His, Asp and Ser (AGY codons).

Evolution of the Genetic Code

Various authors have advanced schemes for the origin of the genetic code based on proposals for direct affinity between amino acids and nucleotide bases or groups of bases. Such affinities are deemed to have played a part in the origin of life in the lifeless 'primitive soup'. The speculations are stimulated by experiments showing the formation of amino acids, purines and pyrimidines from mixtures of methane, hydrogen, ammonia, water, and so on, when treated with electrical discharge or ultraviolet radiation, as reported by Miller, Oro and others^{9,10}. The present genetic code, however, and presumably its immediate antecedents, involve the intervention of adapter molecules of tRNA which combine enzymatically with amino acids, rather than direct affinity between amino acids and nucleic acid molecules.

There are various possibilities for the composition of earlier codes. The number of amino acids coded may have been either more or less than twenty. Amino acids may have been added to the code during evolution or may have

disappeared. I have suggested that arginine displaced ornithine during the evolution of protein synthesis¹¹.

The idea that the number of amino acids in earlier codes was less than twenty is favoured by the fact that some of the 'simple' amino acids, such as serine, glycine and alanine, have four or more codons, and that the codons may be arranged in 'quartets', such as GCU, GCC, GCA and GCG for alanine, in which only the first two bases confer specificity on the amino acid. A code of 'quartets' of codons would suffice for not more than sixteen amino acids, and an arbitrary choice of 'newer' amino acids can be made¹² to reduce the present twenty to fifteen. Perhaps codons were not used for polypeptide chain termination. We shall now consider a different approach to identifying a smaller list of amino acids in the code, emphasising changes in anticodon-codon pairing.

Role of the Nucleotide following the Anticodons

Modifications in this nucleotide at RNA position 40 (ref. 13) are summarised in Table 3. The predominant pattern into which they fall shows that they are evidently concerned with establishing A·U and U·A pairing between the third anticodon position and the first codon position ('3-1 pairing'), occurring on the ribosome during polypeptide synthesis. These modifications enable the composition of a 'primitive' code for ten amino acids, six with eight codons and four with four codons, to be proposed.

Modifications at tRNA position 40 (Table 3) exist in both prokaryotes and eukaryotes. For 3-1 pairing between U and A, the modification in every known case consists of a bulky threonyl-containing side chain on position 6 of adenine. Presumably, these side chains serve, by steric

hindrance, to prevent pairing between U in the third position of the anticodon and G in the first position of the codon. The prevention of this pairing would be essential to fidelity of translation of all codons starting with A with the exception of *E. coli* tRNA^{Met}. This exception supports this hypothesis as follows: this tRNA initiates protein synthesis in *E. coli* by pairing with either AUG (the methionine codon) or GUG (a codon that normally is translated by valine). This tRNA has an unmodified A at position 40. Absence of the modification therefore permits 3-1 pairing by U with A or G and translation of either AUG or GUG as methionine. Uhlenbeck *et al.*⁶ examined the binding of GUG and a number of related oligomers by *E. coli* tRNA^{Met}. GUG and AUG had the same association constants with this tRNA. GUGA, which would bind with the sequence UCAU in the anticodon loop, had a much higher *K* than GUGU, suggesting that GUG in this oligomer was binding to the anticodon.

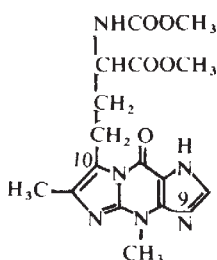
In the case of 3-1 pairing between A and U, the most common modification at position 40 is a bulky isopentyl-containing side chain attached to adenine. An even more bulky molecular grouping, the large and complex 'Y-base', occupies position 40 in the eukaryotic phenylalanine tRNAs (for yeast, wheat and rabbit).

In the case of codons starting with U, it is essential that they should pair with anticodons ending with A and not with anticodons ending with G. These are anticodons for leucine (CUN codons), proline, histidine, glutamine and arginine (CGN codons). The nucleotide adjoining the anticodon in these tRNAs is methylated in prokaryotes (Table 3). Evidently, there is no 3-1 pairing between G and U, but always between G and C. Position 40 is often unmodified in tRNAs for amino acids with anticodons ending in C. This may be because there is no ambiguity in C·G 3-1 pair-

Table 3 Modifications of Nucleosides at Site 40 in tRNAs as Correlated with Recognition of Codons in *E. coli* (*E.c.*) and Yeast (*Ye*)

Codon	Nucleoside adjoining anticodon		Codon	Nucleoside adjoining anticodon		Codon	Nucleoside adjoining anticodon		Codon	Nucleoside adjoining anticodon	
	<i>E.c.</i>	<i>Ye</i>		<i>E.c.</i>	<i>Ye</i>		<i>E.c.</i>	<i>Ye</i>		<i>E.c.</i>	<i>Ye</i>
UUU	ms ² i ⁶ A	Y base	UCU		i ⁶ A	UAY	ms ² i ⁶ A	i ⁶ A	UGU	ms ² i ⁶ A	
UUA	ms ² i ⁶ A		UCA	ms ² i ⁶ A	i ⁶ A	UAA			UGA	—	
UUG	ms ² i ⁶ A	m ¹ G	UCG	ms ² i ⁶ A		UAG			UGG	ms ² i ⁶ A	A
CUU	m ¹ G		CCU			CAY	m ² A		CGU	m ² A, G	A
CUA			CCC								
CUG	m ¹ G		CCA			CAA	m ² A		CGA	m ² A	A
AUU	t ⁶ A		CCG			CAG	m ² A		CGG		
AUA		t ⁶ A	ACU		mt ⁶ A, t ⁶ A	AAU	t ⁶ A		AGU	t ⁶ A	
AUG	t ⁶ A		ACC			AAA	t ⁶ A	t ⁶ A	AGA		t ⁶ A
AUG-f	A	t ⁶ A	ACA			AAG	t ⁶ A	t ⁶ A	AGG		
GUU	A	A†	ACG								
GUA	m ⁶ A	A†	GCU		meI	GAY	m ² A	m ¹ G	GGU	A	
GUG	m ⁶ A		GCA	A	meI	GAA	m ² A		GGA		
			GCG			GAG	m ² A		GGG	A	

Y-base, a guanosine derivative:



m¹G, 1-methylguanosine; meI, methylinosine; m²A, 2-methyladenosine; m⁶A, 6-methyladenosine; mt⁶A, N-methylcarbamoyl in t⁶A; i⁶A = 6-(Δ² isopentenyl)adenosine; t⁶A = N-(9-(β-ribofuranosyl)purin-6-ylcarbamoyl)-L-threonine*; ms²i⁶A, 2-methylthio-6-(Δ²-isopentenyl)adenosine; A†, unidentified adenosine derivative.

* Threonine-containing nucleosides were found in tRNAs accepting arginine and serine.

Table 4 Changes in Ancestral Code Leading to Present Code for Ten Amino Acids

(i) Ancestral Code for Ten Amino Acids

<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>	
NAA } NAG }	UUN	Leu	NGA } NGG }	UCN	Pro	RUA } RUG } YUA } YUG }	UAY	His	NCA } NCG }	UGN	Arg(Orn)
NAG	CUN	Leu	NGG	CCN	Pro	RUG } YUG }	CAY } CAR }	His } Gln }	NCG	CGN	Arg(Orn)
NAU	{ AUN GUN }	Val } Val }	NGU	{ ACN GCN }	Ala	RUU } YUU }	{ AAY GAY }	Asp }	NCU	{ AGN GGN }	Gly
NAC	GUN	Val	NGC	GCN	Ala	RUC } YUC }	GAY } GAR }	Asp } Glu }	NCC	GGN	Gly

(ii) Changes in (i) Leading to Code 2 as a Result of Enzymatic Modification of tRNA Base 40 and Consequent Suppression of U·G Pairing

<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>	
GAA } YAA }	UUY } UUR }	Phe } Leu }	NGA } NGG }	UCN } CCN }	Ser } Pro }	GUA } YUA }	UAY } UAR }	Tyr } C.T. }	NCA } NCG }	UGN } CGN }	Cys } Arg(Orn) }
NAG	CUN	Leu	NGG	CCN	Pro	GUG } YUG }	CAY } CAR }	His } Gln }	NCG	CGN	Arg(Orn)
NAU	AUN	Ile	NGU	ACN	Thr	GUU } YUU }	AAY } AAR }	Asn } Lys }	NCU	AGN	Ser
NAC	GUN	Val	NGC	GCN	Ala	GUC } YUC }	GAY } GAR }	Asp } Glu }	NCC	GGN	Gly

(iii) Changes in (ii) Leading to Present Code as a Result of Reassignment of tRNAs

<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>		<i>a</i>	<i>b</i>	
GAA } YAA }	UUY } UUR }	Phe } Leu }	NGA } NGG }	UCN } CCN }	Ser } Pro }	GUA } YUA }	UAY } UAR }	Tyr } C.T. }	GCA } UCA } CCA }	UGY } UGA } UGG }	Cys } C.T. } Trp }
NAG	CUN	Leu	NGG	CCN	Pro	GUG } YUG }	CAY } CAR }	His } Gln }	NCG	CGN	Arg
GAU } UAU } CAU }	UAY } AUA } AUG }	Ile } Ile } Met }	NGU } NGC }	ACN } GCN }	Thr } Ala }	GUU } YUU }	AAY } AAR }	Asn } Lys }	GCU } YCU }	ACY } AGR }	Ser } Arg }
NAC	GUN	Val	NGC	GCN	Ala	GUC } YUC }	GAY } GAR }	Asp } Glu }	NCC	GGN	Gly

a, Anticodons; *b*, codons, showing anticodon-codon pairing in the first position of codons that includes ambiguity produced by pairing between G and U in addition to G·C and A·U pairing; (ii) code 2 is produced as a result of elimination of this ambiguity; (iii) present code resulting from changes in (ii).

ing, a result which would be consistent with the lack of ambiguity in C·G 1-3 pairing.

The pattern of modification at position 40 in the tRNAs of eukaryotes is less consistent than in prokaryotes. Perhaps eukaryotic ribosomes have assumed some of the functions needed for prevention of ambiguity. It is not known whether the initiator tRNA in eukaryotes pairs with GUG as well as with AUG.

All modifications of nucleotides in tRNA take place by enzymatic mechanisms following transcription. The evolutionary origin of the participating enzymes presumably antedated the prokaryote-eukaryote separation since, for example, t⁶A (Table 3) at position 40 occurs in all *E. coli* and yeast tRNAs that code for amino acids with codons starting with A. In an era before the evolutionary elaboration of these enzymatic modifications of position 40, 3-1 pairing in the ancestral prokaryotes would occur between U·G, and G·U as well as between A·U and U·A. Using only this assumption, and applying it to the present genetic code, an ancestral code results that contains eight codons apiece for each of six amino acids and four codons apiece for each of four amino acids (Table 4). It follows that the identity of these amino acids is such that their codons now start with C or G, and not with A or U. It is necessary, of course, to specify that each set of anticodons, or codons, can specify only one amino acid. As examples, the four codons for valine in the present code can be represented by GUN, pairing with anticodons represented by NAC. U·G pairing would, however, enable the GUN codons also to pair with the four anti-

codons NAU. These anticodons would pair also with the AUN codons. These must have been then assigned to valine, for the same set of anticodons would not specify two different amino acids. The initiator amino acid in protein synthesis by means of AUG and GUG codons would therefore have been valine.

The leucine codons CUN (Table 4) pair with anticodons NAG. The NAG anticodons will, however, also pair (G·U pairing in 3-1 position) with codons UUN, and these will also pair (A·U pairing) with anticodons NAA. Therefore, in this model, the CUN and UUN codons all coded for leucine.

The model for the ancestral code in Table 4 requires, for example, that proline, with anticodons NGG (pairing with codons CCN), should also have anticodons NGA, because NGG and NGA will both pair with codons UCN. Therefore UCN and CCN would be eight codons for proline. The codons with a middle A pose a special problem, for no amino acid has more than two codons with a middle A. It is therefore not possible to select, in terms of the present model, between histidine and glutamine, or between aspartic and glutamic acids, as to which member of each pair should be included in the ancestral code, so all four must be included. The procedure (Table 4, (i)) therefore leads to an ancestral code for ten amino acids.

These can be identified by their present codons all of which start with C or G. Arginine, for reasons advanced elsewhere¹¹, may have been preceded by ornithine. Therefore, ornithine is suggested for the CGN and UGN codons in the

ancestral code. The inclusion of glutamine is surprising: this amino acid is formed enzymatically from glutamic acid. An alternative possibility is that all RUG, YUG, RUA and YUA anticodons belonged to histidine, and that the divergence between histidine and glutamine occurred during the later stage represented by Table 4 (ii).

It is of interest that yeast leucine-3 tRNA, with the anticodon CAA, pairing with UUG has 1-methylguanosine, that is, only a small side chain rather than an isopentenyl side chain, at position 40. This seeming anomaly is, however, consistent with the fact that specific distinction is not required between UUR and CUN codons, all six of which are for leucine (Table 4 (ii)). Therefore position 40 may have discarded 'bulky' modifications during the evolution of eukaryotic leucine tRNAs for pairing with codons starting with U.

The ten amino acids in the ancestral code would be adequate for the synthesis of proteins with reasonably versatile properties, except that the absence of serine is surprising. The list includes leucine and valine with hydrophobic side chains; the 'helix-breaker', proline; the two simple amino acids, alanine and glycine; histidine, which binds substrates and prosthetic groups; aspartic and glutamic acids, the acidic pair; a basic amino acid, ornithine; and glutamine, which is somewhat hydrophilic. According to the model, serine entered the code with eight codons, UCN and AGN, at the intermediate stage.

The expansion of the code to eighteen amino acids (Table 4 (ii)) was preceded by modifications of tRNA base 40, leading to the suppression of 3-1 pairing between G and U, and U and G. This enabled modifications of the amino acid recognition characteristics, by mutational base changes, to be adopted in tRNAs, so that 'new' amino acids entered

the process of protein synthesis, thus conferring novel and advantageous properties on proteins. Further expansion of the code to twenty amino acids then took place by other reassignments of tRNA molecules as summarised in Table 4 (iii). It is customary to postulate that a single species of organism, possessing the present code, displaced all other living organisms and proceeded, by divergent evolution, marked by gene duplication, allopatric speciation and natural selection, to give rise to various lines of descent that led to the existing terrestrial biota.

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- ¹ Loftfield, J., *Proc. Nucl. Acid. Res. molec. Biol.*, **12**, 87 (1972).
- ² Hill, C. W., Squires, C., and Carbon, J., *J. molec. Biol.*, **52**, 557 (1970).
- ³ Squires, C., and Carbon, J., *Nature new Biol.*, **233**, 274 (1971).
- ⁴ Jukes, T. H., and Holmquist, R., *Biochem. biophys. Res. Commun.*, **49**, 212 (1972).
- ⁵ Crick, F. H. C., *J. molec. Biol.*, **19**, 548 (1966).
- ⁶ Uhlenbeck, O., Baller, J., and Doty, P., *Nature*, **225**, 508 (1970).
- ⁷ Nishimura, S., *Prog. Nucl. Acid. Res. molec. Biol.*, **12**, 49 (1972).
- ⁸ Kammen, H. O., and Spengler, S. J., *Biochim. biophys. Acta*, **213**, 352 (1970).
- ⁹ Miller, S. L., *Biochim. biophys. Acta*, **23**, 480 (1957).
- ¹⁰ Oro, J. J., in *Current Aspects of Exobiology*, chap. 1 (Pergamon, New York, 1965).
- ¹¹ Jukes, T. H., *Biochem. biophys. Res. Commun.*, **53**, 709 (1973).
- ¹² Jukes, T. H., *Molecules and Evolution*, 1 (Columbia University, New York, 1966).
- ¹³ Holmquist, R., Jukes, T. H., and Pangburn, S., *J. molec. Biol.*, **77**, 91 (1973).
- ¹⁴ Celis, J. E., Hooper, M. L., and Smith, J. D., *Nature new Biol.*, **244**, 261 (1973).

LETTERS TO NATURE

PHYSICAL SCIENCES

Satellite Observations of Intensity Variations of the Zodiacal Light

OBSERVATIONS of the intensity of the zodiacal light at 6530 Å have been made with a photometer on board the satellite D2A 'Tournesol'. This satellite was launched on April 15, 1971, from Kourou (French Guiana) into an orbit of inclination 46° with a perigee of 456 km and an apogee of 700 km. The spin axis was oriented towards the Sun with an accuracy of ~15 arc min and a spin period of 60 s (Fig. 1). The line of sight of the photometer is perpendicular to the spin axis and therefore scans the celestial sphere in a plane orthogonal to the ecliptic plane and to the direction of the Sun. Observations in a plane perpendicular to the Earth-Sun line (that is, at elongation $\epsilon=90^\circ$ or $\epsilon=-90^\circ$) were carried out during the night phase, when the solar depression angle was $>25^\circ$ and the altitude of the lowest observed points was >135 km. Because of this, the signal contained no contribution from the night-glow, but was only due to zodiacal light and starlight. The data cover, without interruption, the period from April 19, 1971, to June 1973.

The photometer has been described previously¹. It has one channel at Balmer alpha (6563 Å), and a second at 6530 Å. Only 6530 Å data will be discussed here. The

bandwidth was 20 Å and the signal was integrated for $\frac{2}{3}$ s. Because of this integration time the field of view is extended from $1^\circ 20' \times 2^\circ 40'$ to $6^\circ 35' \times 2^\circ 40'$. A signal

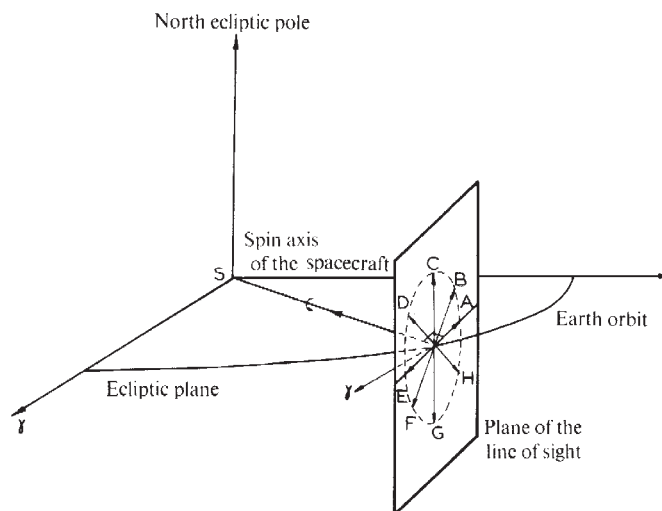


Fig. 1 Line of sight of the photometer. A, $\epsilon=90^\circ$, $\beta=0^\circ$; B, $\epsilon=90^\circ$, $\beta=45^\circ$; C, $\epsilon=90^\circ$, $\beta=45^\circ$; D, $\epsilon=90^\circ$, $\beta=45^\circ$; E, $\epsilon=-90^\circ$, $\beta=0^\circ$; F, $\epsilon=-90^\circ$, $\beta=-45^\circ$; G, $\epsilon=-90^\circ$, $\beta=-45^\circ$; H, $\epsilon=90^\circ$, $\beta=-45^\circ$. ϵ is $\pm 1^\circ 20'$; β is $\pm 3^\circ 20'$.

of 100 S_{10} (vis) provides a count rate of ~ 670 counts s^{-1} , compared with a dark current of 60 counts s^{-1} outside the South Atlantic anomaly. A star takes two days to cross the field of view because of the rotation of the Sun-spacecraft line. This allowed us to demonstrate that after taking into account variations of sensitivity across the field of view, no sensitivity change could be detected. Furthermore, the on-board calibration source gave a signal that was constant within $\pm 5\%$. We therefore believe that no short term fluctuations of the instrument response occurred during the 2 yr of observation. The instrumental arrangement thus allows a measurement of the zodiacal light and its variation from day to day.

Table 1 Points Ignored in Editing Data

Moonlight (arbitrary units)	New Moon					Quar- ter Moon						Full Moon
θ	0	1	2	3	4	5	6	7	8	9	10	
(Line of view, Moon direction)	25°	25°	25°	30°	40°	50°	60°	60°	70°	70°	180°	

All data relevant to a field of galactic latitude less than 30° , or containing a known star of magnitude brighter than +6 or corresponding to the simultaneous observation on the 6563 Å channel of an HII region or of a non-nominal geocoronal Balmer α emission have been removed from this analysis. All points less than an angle θ from the Moon, given in Table 1, together with the Lagrangian points of the Earth-Moon system have also been removed. After this editing of the data, the recorded signal N was generally between 600 and 3×10^3 counts s^{-1} . The zodiacal light intensity is $I = (N - n)c/20 - h(l^{II}, b^{II})$ where n is the dark current, c the absolute calibration coefficient and $h(l^{II}, b^{II})$ the integrated starlight contribution² as a function of galactic coordinates. Two consecutive years of data have already been reduced.

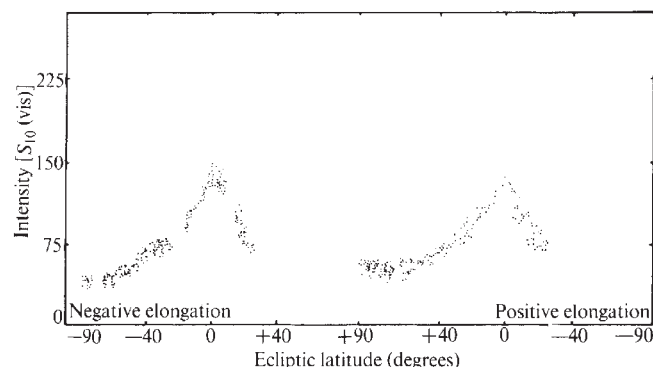


Fig. 2 Typical example of the 6530 Å intensity as a function of the ecliptic latitude β for May 25, 1971.

Figure 2 shows a typical example of the 6530 Å intensity measured as a function of the ecliptic latitude β for May 25, 1971. The measurements were taken over four nocturnal parts of orbits and about sixty rotations of the satellite around its axis. The intensity has two maxima as the line of sight intersects the ecliptic plane and two minima in the direction of the ecliptic poles. The small scatter of the data allows an intensity of the zodiacal light to be measured quite accurately. As was found using OSO-2 satellite data³ the zodiacal light intensity remained constant during successive orbits on one day.

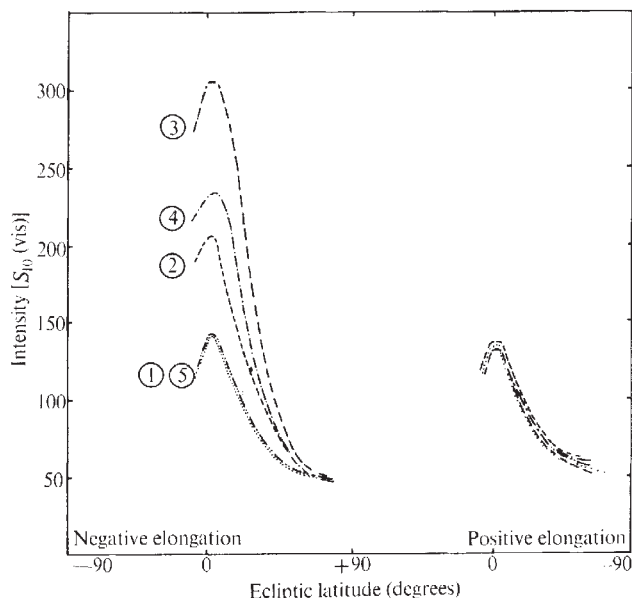


Fig. 3 Variation of the intensity of the zodiacal light. 1, November 4, 1971; 2, November 6, 1971; 3, November 7, 1971; 4, November 10, 1971; 5, November 12, 1971. Full Moon on November 2, 1971.

The intensity of the zodiacal light was sometimes observed (contrary to other satellite observations^{4,5}) to vary from one day to the next. Figure 3 shows an example of the variation of the intensity as a function of ecliptic latitude from November 5 to November 11, 1971. There was an increase of about 100% in the intensity for negative elongations, compared with the values on November 4 and November 12, while for the positive elongations it remained constant. To check whether the enhancements of the intensity were due to an asymmetry in the observations, measurements on November 4 were compared with those on November 7 under the same conditions: spacecraft latitude $\sim 45^\circ$, local time near midnight, no stray emissions, line of view above the horizontal plane of the spacecraft, and galactic latitudes comparable. Under those conditions, the enhancement was $\sim 100\%$.

Table 2 Comparison with Previous Measurements

β	Zodiacal light intensity at 90° elongation (S_{10} (vis))			
	Roach <i>et al.</i> ⁶	Dumont ⁷	Lillie ⁸	Our results
0°	250	202 to 170 *	180	150 ± 10
45°	140	90 to 62 *	75	75 ± 5
90°	110	65 to 50 *	50	57 ± 5

* Lowest values.

To study the annual variation, the mean value of the intensities for eight directions (Fig. 1) (in the ecliptic plane, towards the ecliptic poles and at $\pm 45^\circ$ latitude) was computed for each day. The r.m.s. value was about $7S_{10}$ (vis). The number of measurements in each field is about fifteen, and the removal of a large proportion of data (to avoid stray emissions) is responsible for the fact that the eight mean values do not exist simultaneously on Fig. 4. When there is no increase, our results are in good agreement with previous measurements as is shown in Table 2. The main features of the data are the increases in intensity of up to 100% in a few days with a time constant of 3 to 25 d. The increases do not always appear simultaneously in all directions but may occur over a limited part of the sky.

The observations at the ecliptic poles are of considerable interest. Figure 5, which represents two consecutive years

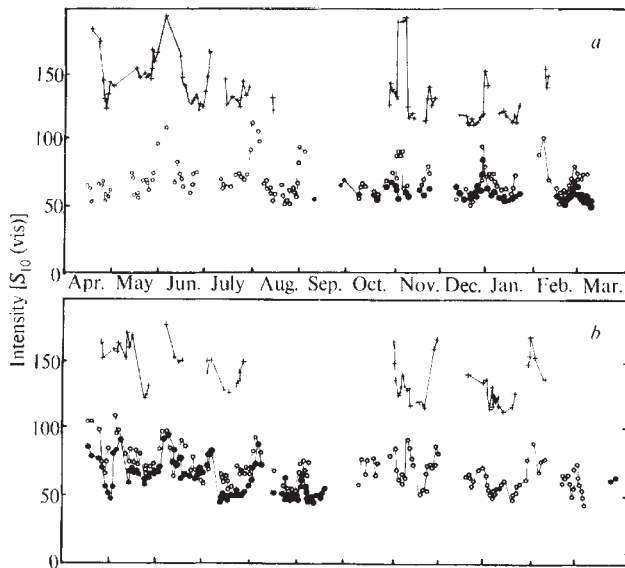


Fig. 4 Mean value of the intensity for eight directions (see Fig. 1). *a*, $\epsilon < 0$: +, ecliptic plane (E); $\circ$, $\pm 45^\circ$ latitude (D, F); $\bullet$, south pole (G). *b*, $\epsilon > 0$: +, ecliptic plane (A); $\circ$, $\pm 45^\circ$ latitude (B, H); $\bullet$, north pole (C).

of data, shows parallel trends of the evolution with time of the ratio of polar intensity to the intensity at 45° , and definitely proves that the variations of the zodiacal light are correlated with the position of the Earth in its orbit.

These variations may explain why the brightness of the zodiacal light is uncertain⁹ by a factor of ~ 2 . The large value of the variations and their dependence on angle of sight rule out an effect of the solar wind or of the solar activity.

In the example presented in Fig. 3, a 100% increase in intensity was observed near the ecliptic plane on November 7 and there was an enhancement of more than 50% for 6 d around the maximum. The Earth might not at that time have been far from the debris of comet Biela (Andromedids), the inclination of which to the ecliptic is 6° .

On April 21, an increase of 50% was observed in the polar and 45° directions and the half enhancement (25%) had been detected for 4 d. During this period the Earth encountered the Lyrids, the debris of comet 1861-I, the inclination of which is 80° .

It thus seems that the zodiacal light fluctuation by a factor of 2 is due to the scattering of solar light by local streams of particles. The variations in the increases as a function of angle to the ecliptic poles depend on the orbits of the particles. The magnitude of the increase would seem to indicate that the zodiacal cloud consists of a collection of such streams created on account of the Poynting-Robert-

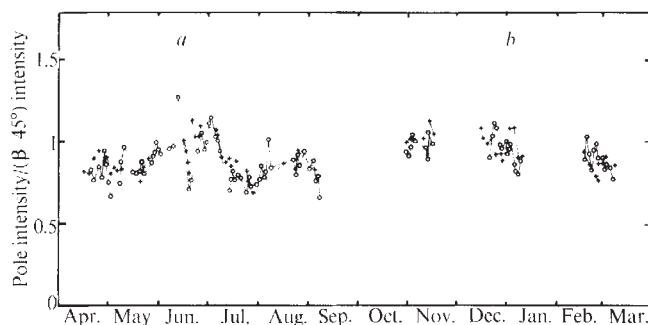


Fig. 5 Observations at the ecliptic poles in two consecutive years. $\circ$, April 1971 to March 1972; +, April 1972 to March 1973. *a*, North pole side; *b*, south pole side.

son effect by rather recent comets with a short lifetime. The interweaving of these elliptic toruses mainly constitutes the zodiacal cloud.

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- ¹ Levasseur, A. C., and Blamont, J. E., *J. geophys. Res.*, **78**, 3881 (1973).
- ² Roach, F. E., and Megill, L. R., *Astrophys. J.*, **133**, 228 (1961).
- ³ Gillett, F. C., *The Zodiacal Light and the Interplanetary Medium* (edit. by Weinberg, J. L.), NASA Special Publication, **150**, 9 (1967).
- ⁴ Sparrow, J. G., and Ney, E. P., *Astrophys. J.*, **154**, 783 (1968).
- ⁵ Sparrow, J. G., and Ney, E. P., *Science, N.Y.*, **181**, 438 (1973).
- ⁶ Smith, L. L., Roach, F. E., and Owen, R. W., *Planet. Space Sci.*, **13**, 207 (1965).
- ⁷ Dumont, R., and Sanchez-Martinez, F., *Astr. Astrophys.*, **22**, 321 (1973).
- ⁸ Lillie, C. F., *The Scientific Results from OAO-2* (edit. by Code, A. O.), NASA Special Publication, **310**, 109 (1971).
- ⁹ Roosen, R. G., *Rev. Geophys. Space Phys.*, **9**, 275 (1971).

New Galactic Supernova Remnants

WE have identified twenty-seven galactic radio sources as supernova remnants by comparing 408 MHz observations from the Molonglo radio telescope and 5,000 MHz observations of comparable resolution from the Parkes 64-m radio telescope. The distribution of these and other supernova remnants with positions estimated from a new surface brightness-linear diameter relation has been investigated for evidence of galactic spiral structure.

Most extended non-thermal radio sources close to the galactic plane have morphological and spectral characteristics similar to those of the unambiguously identified galactic supernova remnants, and are consequently also considered to be supernova remnants (SNRs). These characteristics include: (i) a non-thermal radio spectrum, due to radiation by the synchrotron mechanism invoked to describe radio emission from SNRs; (ii) where resolution permits such recognition, a 'ring' or 'part-ring' radio brightness distribution indicative of an expanding shell source.

Within the region surveyed, seventy sources have previously been classified as SNRs (see ref. 1). Therefore the new observations represent a significant increase in the number of galactic SNRs observed at southern declinations, and in particular of older low surface brightness remnants for which previous lists were incomplete.

Green² has completed a high resolution 408 MHz galactic survey using the Molonglo telescope, which has a beamwidth of $2.86'$ in right ascension by $2.86'$ (zenith angle) in declination. The region with new galactic coordinates $|b| < 3^\circ$ and l $195^\circ - 360^\circ - 55^\circ$ was observed. Because at 408 MHz non-thermal radiation predominates, the survey proved particularly useful for work on SNRs.

We prepared a list of those extended galactic sources from the Molonglo survey which had not previously been classified on the basis of their spectra and structure as HII regions, SNRs, or extragalactic radio sources. A cursory estimate of the spectral index was obtained using the 2,700 MHz flux densities from the Parkes survey (see ref. 3 and references therein); however, accurate spectral indices can best be obtained from investigations at two widely different frequencies with similar beamwidths. Accordingly, some seventy sources

which had been noted by Green² as worth investigating as possible SNRs from the 408 MHz–2,700 MHz comparison in the range l 260°–345°, plus some additional suspects outside this range, were observed with the Parkes 64-m radio telescope at 5,000 MHz (half-power beamwidth ~ 4 arc min). From the 408 MHz–5,000 MHz comparison we have been able to derive accurate positions, flux densities, spectral indices, and angular sizes for twenty-seven of these sources believed to be SNRs. Brightness temperature contour maps for the sources are in preparation, and will be presented separately.

For the 408 MHz observations the flux density scale used was that of Wyllie⁴. During each observing session several standard sources selected from Hundstead^{5,6} were measured for calibration purposes. At 5,000 MHz the system was calibrated using Hydra A for which a flux density $S(5,000 \text{ MHz})$ of 13.5 f.u. was assumed.

The derived parameters for the new remnants are given in Table 1, which includes the galactic source number ($l-b$), the equatorial coordinates (1950), the mean angular diameter and the flux densities and spectral index estimate for each source. The mean angular diameters $\langle\phi\rangle = (\phi_{\text{RA}} \times \phi_{\text{Dec}})^{1/2}$ were estimated from the outer radio isophotes used to determine the limits of integration in the estimation of the flux densities, and not from the half-power levels often used. The spectral index α is defined by the simple power law $S_\nu \propto \nu^\alpha$, where S_ν is the flux density at frequency ν . The flux densities for both 408 MHz and 5,000 MHz observations have errors due to noise and calibration uncertainties of less than 5%. The corresponding error in spectral index may be greater in confused regions where the extent of the source is not clearly defined. In such cases particular care was taken in defining the extent of integration. Sources with relatively flat spectra have been retained only where a thermal spectral index of -0.1 is believed to lie outside the range of possible errors. To exclude extragalactic objects, sources with relatively steep spectra have been excluded unless they show a shell structure.

Some properties of individual sources are as follows:

G321.9–0.3. This SNR extends into the error box of the Uhuru X-ray source⁷ 2U1516–56. But since the X-ray source is pulsating it is unlikely that the SNR itself is the origin of the X-ray emission.

G323.5+0.1. Since this non-thermal source is confused at 5,000 MHz with a neighbouring HII region, it has only been possible to place limits on the 5,000 MHz flux density.

G335.2+0.1. The proximity to the X-ray source 2U1624–49 may be worth further investigation.

G339.2–0.4. Although the spectrum of this source is not as steep as average ($\alpha_{\text{av}} = -0.48$) it seems to be non-thermal and has a part-shell structure. Its proximity to the X-ray source 2U1641–45 and the recently discovered Parkes pulsar 1641–45 is of interest.

If the relation between surface brightness Σ and linear diameter D for SNRs at known distances is established, the distances and galactic distribution of all others may be investigated if they are treated as being identical. Representing the relation as $\Sigma \propto D^{-n}$, earlier investigations of galactic SNRs have yielded empirical values of n in the range 2.7 (at 400 MHz, ref. 8) to 4.5 (at 1,000 MHz, ref. 1). Spectral and evolutionary differences, as well as observational errors, would be expected to have contributed to the uncertainty in n .

Mathewson and Clarke⁹ used fourteen SNRs detected in the Magellanic Clouds to establish the relation

$$\Sigma_{408} = D_{\text{pc}}^{-3} 10^{-15} \text{ W m}^{-2} \text{ Hz}^{-1} \text{ sr}^{-1}$$

This relation has the advantage that the distance of all the calibrators is reliably known, whereas for many of the galactic calibrators uncertainties in their distances of up to 50% exist. But as noted by Mathewson and Clarke⁹ the Magellanic Cloud SNRs seem to be inherently brighter objects than galactic SNRs of the same linear diameter.

In view of the uncertainties in earlier galactic Σ – D relations, and the apparent difference between Galactic and Magellanic Cloud SNRs, the galactic Σ – D relation has been rederived. The distance calibrators used were those class 1 and class 2 calibrators listed by Ilovaisky and Lequeux¹⁰ with surface brightness values in the range 3×10^{-21} to $3 \times 10^{-19} \text{ W m}^{-2} \text{ Hz}^{-1} \text{ sr}^{-1}$, this being the range over which we wish to apply the relation for distance determination. (We note that this excludes Cas A, the Crab, and Cygnus Loop.) The surface brightness results for the southern sources were calculated from the Molonglo survey.

Table 1 Parameters for the 'New' Remnants

Galactic source number	Position ($\sim$ centroid)		Mean angular diameter $\langle\phi\rangle$ (arc min)	Flux densities (f.u.)		Spectral index α	Distance d (kpc)	Displacement from galactic plane z (pc)
	RA (1950)	Dec. (1950)		408 MHz	5,000 MHz			
293.8+0.6	11 33 00	–60 36	19.4	9.0	2.1	–0.58	10.2	+107
296.0–0.6	11 48 15	–62 27	17.2	6.9	0.74	–0.89	11.6	–121
299.0+0.2	12 15 05	–62 11	21.3	12.6	4.7	–0.39	8.8	+31
302.3+0.7	12 42 40	–61 51	16.8	7.5	3.0	–0.36	11.3	+138
308.7+0.0	13 38 05	–62 01	12.3	16.7	7.0	–0.35	9.6	0
309.2–0.6	13 42 30	–62 37	12.8	10.0	3.9	–0.37	11.3	–118
309.8+0.0	13 47 05	–61 50	21.2	26.4	7.4	–0.51	6.9	0
315.4–0.3	14 32 00	–60 22	20.4	17.1	4.9	–0.50	8.1	–42
321.9–0.3	15 16 45	–57 29	24.8	18.3	7.8	–0.34	7.4	–38
323.5+0.1	15 25 05	–56 13	9.7	4.2	0.5 < S < 2.0	–0.8 < α < –0.3	16.6	+29
327.2–1.0	15 50 35	–54 56	16.3	10.6			10.2	–178
330.2+1.0	15 57 20	–51 25	11.1	8.6	4.0	–0.30	12.4	+216
335.2+0.1	16 23 50	–48 36	18.6	27.1	8.6	–0.46	7.2	+12
337.2–0.7	16 35 45	–47 45	8.4	3.8	0.70	–0.67	17.9	–218
339.2–0.4	16 41 50	–46 03	10.8	7.5	4.5	–0.20	13.2	–92
340.4+0.4	16 43 00	–44 34	5.9	8.2	2.9	–0.41	15.4	+107
340.6+0.3	16 44 05	–44 30	4.1	7.0	2.8	–0.36	18.6	+97
344.7–0.1	17 00 15	–41 35	8.1	4.7	1.3	–0.51	16.9	–29
346.6–0.2	17 06 40	–40 06	12.3	14.9	4.3	–0.49	10.0	–35
350.0–0.3	17 17 35	–37 24	9.0	10.7	1.7	–0.73	12.4	–65
350.0–1.8	17 23 50	–38 18	37.9	49.5	13.6	–0.51	4.6	–144
351.2+0.1	17 19 05	–36 08	13.0	8.1	3.1	–0.38	12.1	+21
352.7–0.1	17 24 20	–35 05	11.9	9.6	2.3	–0.57	11.8	–20
355.9–2.5	17 42 30	–33 43	15.8	12.3	3.4	–0.51	9.8	–427
11.4–0.1	18 07 55	–19 06	13.6	5.3	2.8	–0.25	13.7	–24
15.9+0.2	18 16 05	–15 02	10.7	7.7	1.9	–0.56	13.1	+45
24.7–0.6	18 35 40	–07 36	16.7	12.3	3.6	–0.49	9.6	–100

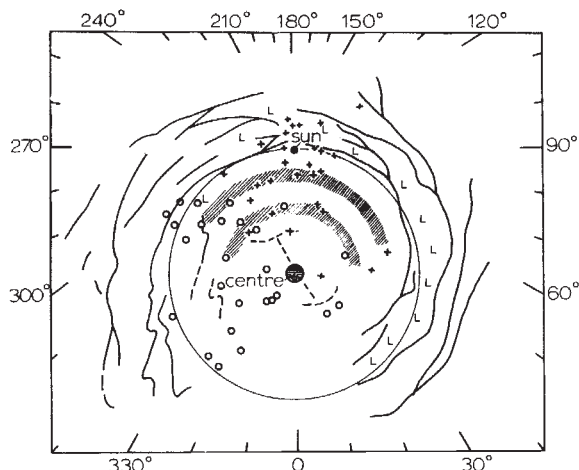


Fig. 1 Galactic neutral hydrogen map of Kerr¹¹, with SNR positions superimposed. The new SNRs are indicated by circles, and those from Woltjer¹² by crosses.

The data are well fitted by either of the expressions

$$\Sigma_{408} = 0.7 D_{\text{pc}}^{-3.0} 10^{-15} \text{ W m}^{-2} \text{ Hz}^{-1} \text{ sr}^{-1} \quad (1)$$

or

$$\Sigma_{408} = D_{\text{pc}}^{-3.11} 10^{-15} \text{ W m}^{-2} \text{ Hz}^{-1} \text{ sr}^{-1} \quad (2)$$

In expression (1) the Mathewson and Clarke exponent was retained. The difference in surface brightness could then indicate, as Mathewson and Clarke suggest, a mean initial energy for the Magellanic Cloud SNRs about 25% greater than that for galactic SNRs. In expression (2) the data were fitted retaining the Mathewson and Clarke proportionality constant and altering the exponent to fit the data, which might indicate a difference in evolutionary behaviour. But the difference in distance estimates for the two expressions over the Σ range of interest is less than 3%. We have arbitrarily adopted expression (1) for our subsequent calculations. SNR distance estimates thus obtained are about 10% less than those obtained using the Mathewson and Clarke relation, but somewhat greater than distance estimates obtained using earlier Σ - D relations. The distance estimates d , and displacement from the galactic plane z , for each of the newly discovered SNRs are included in Table 1.

The galactic distribution of the new SNRs catalogued in Table 1 is shown in Fig. 1 superimposed on the neutral hydrogen spiral arm map of Kerr¹¹. Kerr shows the two major arms within the solar circle (the Sagittarius and Norma-Scutum arms) as only roughly defined in view of the uncertainties in kinematic distances for this region. The position estimates of the new SNRs are indicated by circles. Also included on the diagram and indicated by crosses are the position estimates found using the new Σ - D relation, of the well known SNRs catalogued in Tables 1 and 3 of Woltjer¹² (with the exception of G8.5-0.3, which has been shown to be thermal¹³). The Crab, Cas A, and the Cygnus Loop are not included in the diagram since they have surface brightness values outside the limits used in deriving the Σ - D relation. SNRs with $|z| > 250$ pc have also been excluded from the diagram. Flux densities used in the distance calculations for the southern sources were taken from the Molonglo survey, while for those sources north of declination $+19^\circ$ data were taken from Woltjer¹².

The SNR distribution exhibits certain features similar to the HI distribution. Most of these SNRs lying between the Sun and galactic centre appear to lie on the inner spiral arms. The group of SNRs approximately 3 to 4 kpc from the galactic centre may indicate the position of the inner expanding arm (shown as dashed in Fig. 1).

More reliable distances for known SNRs are desirable in order to investigate the intrinsic dispersion of the ϵ - D evolu-

tionary curve, and to see whether the technique of using SNRs as spiral arm tracers can be pursued further.

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¹ Milne, D. K., *The Crab Nebula*, I.A.U. Symposium No. 46, 248 (1971).

² Green, Anne J., thesis, Univ. Sydney (1972).

³ Day, G. A., Caswell, J. L., and Cooke, D. J., *Austr. J. Phys., Astrophys. Suppl.*, **25**, 1 (1972).

⁴ Wyllie, D. V., *Mon. Not. R. astr. Soc.*, **142**, 229 (1969).

⁵ Hundstead, R. W., *Mon. Not. R. astr. Soc.*, **152**, 277 (1971).

⁶ Hundstead, R. W., *Mon. Not. R. astr. Soc.*, **157**, 367 (1972).

⁷ Uhuru Catalogue of X-ray Sources, *Astrophys. J.*, **178**, 281 (1972).

⁸ Poveda, A., and Woltjer, L., *Astr. J.*, **73**, 65 (1968).

⁹ Mathewson, D. S., and Clarke, J. N., *Astrophys. J.*, **180**, 275 (1973).

¹⁰ Ilovaisky, S. A., and Lequeux, J., *Astr. Astrophys.*, **18**, 169 (1972).

¹¹ Kerr, F. J., *The Spiral Structure of our Galaxy*, I.A.U. Symposium No. 38, 95 (1970).

¹² Woltjer, L., *A. Rev. Astr. Astrophys.*, **10**, 129 (1972).

¹³ Dickie, J. R., and Milne, D. K., *Austr. J. Phys.*, **25**, 539 (1972).

Are Large Time Differences in Meteorite Formation Real?

IN recent years two types of cosmochronological dating have been interpreted to indicate that there are large time intervals, a few times 10^7 yr, between the formation of various meteorite samples. For example, extrapolation backwards along isochrons in seven basaltic achondrites led Papanastassiou and Wasserburg¹ to establish a 'standard' initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.69898 ± 0.00003 (BABI $\equiv$ 'basaltic achondrite best initial'). Similar isochrons in another basaltic achondrite, Angra dos Reis, led, however, to an initial ratio of 0.69884 ± 0.0004 (ADOR $\equiv$ 'Angra dos Reis')². The difference between these two values is attributed to the accumulation of different amounts of the ^{87}Sr decay product of ^{87}Rb . If this reflects a difference in the times of fractionation between Rb and Sr in the meteoritic material, then the time interval is 14×10^6 yr. More recently, Tatsumoto, Knight, and Allegre³ found significant differences in the radiogenic $^{207}\text{Pb}/^{206}\text{Pb}$ ratios in Angra dos Reis and in two other basaltic achondrites, the value for Angra dos Reis being 0.6197 and the average of the other two being 0.6081. These nuclides result from the decay of ^{235}U and ^{238}U . If the differences are attributed to different times for the events of lead-uranium fractionation in the meteoritic materials, then the indicated time interval is 27×10^6 yr.

Are these time differences real? Or were there significant isotopic abundance variations in different parts of the primitive solar nebula in which different meteorite samples accumulated?

These questions are of obvious importance in establishing boundary conditions on the history of the Solar System. I present here a case for the second alternative and suggest experiments to settle the issue.

The isotopic composition of the heavier elements can vary if there is a variable ratio of s-process products to r-process products. These have different astrophysical sources. The s process probably takes place in stars somewhat more massive than the Sun while they are in their more advanced stages of evolution in the red giant region of the Hertzsprung-Russell diagram⁴. The products are probably ejected into space relatively gently in stellar winds or as parts of planetary nebulae; they cannot withstand unaltered the severity of a supernova environment. The r process probably occurs in the envelopes of type II supernovae when the high ion temperature precursor of the shock wave becomes strong enough to cause helium spallation, with copious neutron formation and ultimate production of deuterium⁵⁻⁷. The presupernovae of type II seem to be stars of at least $4 M_{\odot}$. In the following discussion I shall assume that the s-process products are uniformly distributed in space; to the extent that this may not be true, the effects discussed will be amplified.

Reeves⁸ has recently applied the analysis of Spitzer⁹, of the slowing down of supernova debris through interaction with the interstellar medium, to a discussion of the thoroughness of the mixing of the supernova products throughout the interstellar medium. A type II supernova will probably interact dynamically with about $10^4 M_{\odot}$ of gas in slowing down in about 10^6 yr. Reeves assumes that the supernova nucleosynthesis products are intimately mixed with this material, and that mixing with additional material will continue during the course of a few more million years as a result of random motions of the gas. On this basis he concludes that any typical part of the gas will be contaminated with fresh supernova debris every few million years, and that the isotopic composition of the interstellar medium will be maintained the same over a large local volume of the Galaxy.

This analysis does not, however, take into account the effects of the interstellar magnetic field. Even in typical neutral hydrogen clouds the magnetic field is effectively tied to the gas because of ions produced by ultraviolet radiation. Some mixing of supernova debris across field lines may take place as a result of short-scale dissipation processes, particularly during the early part of the supernova envelope expansion. A typical type II supernova explosion is likely to eject only a few solar masses of material, and this may perhaps be mixed into only about $10^2 M_{\odot}$ of the $10^4 M_{\odot}$ which participate in the dynamics of the slowing down process. Because of the operation of Rayleigh-Taylor instabilities, the supernova debris is likely to end up in the form of many thin tubes of magnetic flux threading through the much larger amount of material which has participated in the dynamical deceleration. Subsequent mixing would be mainly confined to longitudinal diffusion along the field lines, a very slow process.

There are about $10^{10} M_{\odot}$ of gas in the Galaxy, and about one type II supernova explosion per 10^2 yr. Thus it may be that any given part of the interstellar medium has received a direct injection of the debris from only about one of these supernovae.

Processes do, however, exist which should ultimately lead to complete mixing of material. Material in very dense interstellar clouds is shielded from ultraviolet light, so that significant drifting across field lines may occur, particularly for elements with higher ionization thresholds. Under these circumstances, heavier atoms can also adhere to interstellar grains, which can drift across field lines more easily than ions. Occasionally interstellar material will participate in a star formation event, in which $\sim 10^4 M_{\odot}$ collapse to high density with fragmentation to form stars in what become expanding associations. The condition that a newly-formed association of stars should be unbound is that more than half of the collapsed mass should be ejected, presumably as gas which never

became part of the stars. Since the interstellar magnetic field can slip out of the collapsing gas¹⁰ when the density exceeds 10^6 atoms cm^{-3} and since the collapsing gas is likely to be turbulent, thorough mixing will occur at that time. Truran and Cameron¹¹ have estimated that about half the interstellar medium had been through stellar interiors at the time of formation of the Solar System. From these various considerations I estimate that the products of stellar nucleosynthesis probably become homogenised in the interstellar medium after about 3×10^9 yr, but that more recent products remain localised in thin tubes of magnetic flux.

Although it seems that the production of the more abundant lighter elements was concentrated toward the early history of the Galaxy, the s and r processes are of secondary and tertiary character, transforming previous products of nucleosynthesis. Thus for the present purposes it is sufficient to suppose that the heavy elements have had a uniform production rate throughout galactic history. Thus at the time of formation of the Solar System, the interstellar cloud complex which was about to collapse probably had about 80% of the heavy elements uniformly distributed throughout the mass, on which was superposed a highly local fluctuation in abundance of the order of 25% of the average abundance.

During the collapse, turbulent mixing would tend to reduce these local fluctuations¹². After fragmentation, the gas would settle into differentially rotating flattened gaseous disks, which would then dissipate to form stars. Differential rotation would tend to homogenise composition at any given radius, but some small radial composition differences might persist. This might be the source of the strontium isotopic differences characteristic of BABI and ADOR, and the related lead-lead differences. In the following I assume that there was a fractional difference of Δ in the r-process abundances in BABI and ADOR, and enquire what effects this would have on strontium and uranium isotopic abundances.

The strontium case is actually very complicated. ^{86}Sr and ^{87}Sr are s-process isotopes, except for the small leakage of the r process to the latter through ^{87}Rb , which has a half-life four times the age of the Galaxy. Because mass spectrometers sometimes suffer from mass fractionation problems in the ion source, however, the common practice is to refer the abundance of ^{87}Sr to the slope of abundances between ^{86}Sr and ^{88}Sr . The latter isotope has both s and r-process components. Furthermore, the s-process neutron capture path passes through ^{85}Kr , which has a half-life of 10.76 yr. A recent analysis by Scalo and Ulrich⁴ indicates that the s process may occur in a series of relatively short neutron bursts. So the s-process path probably divides at ^{85}Kr , producing both ^{86}Sr and ^{86}Kr . By itself, this may introduce some fluctuations in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio, but I shall ignore this complication here. One cannot, however, take the abundance of ^{86}Kr as indicative of the local r-process abundance.

The other neighbouring r-process-only isobars are ^{82}Se , ^{96}Zr and ^{100}Mo , with abundances of 6.18, 0.784 and 0.385, respectively, on an abundance scale¹³ where silicon = 10^6 . An interpolation suggests the r-process abundance contribution to ^{88}Sr to be about 2.5 out of a total abundance of 22.2. A fractional abundance fluctuation Δ in this contribution would thus produce a fluctuation $0.5 (2.5/22.2) \Delta = 0.056 \Delta$ in the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio that would be inferred from analysis of mass spectrometer measurements. The fractional difference between BABI and ADOR of 2.0×10^{-4} would thus imply $\Delta \approx 3.5 \times 10^{-3}$. This estimate must be regarded as uncertain by a factor three. Nevertheless the fluctuation represents a reduction by two orders of magnitude from the local fluctuations that may have characterised that part of the interstellar medium which collapsed to form the Solar System.

The analysis of the uranium isotopes must be even cruder because of their radioactive character. The fractional abundance of a radioactive isotope, relative to the total amount ever formed, is the ratio of its mean life to the total time needed to accumulate a non-radioactive nuclide at the recent production

rate. The mean life of ^{238}U is 6.4×10^9 yr, or twice the time required for homogenisation of abundances. The homogenised background of this nuclide may thus be about 50% of the total, with local fluctuations in abundance of the order of a factor two. The mean life of ^{235}U is 1.02×10^9 yr. The homogenised background abundance level is thus only about 4% of the average, and local fluctuations may be an order of magnitude about the mean. There will be some correlations between the abundance fluctuations of ^{235}U and ^{238}U . I estimate the fluctuations that may occur in the radiogenic $^{207}\text{Pb}/^{206}\text{Pb}$ ratio to be of order 20Δ , but not even the sign of these can be predicted, because a region with excess r-process production might or might not have had a recent contribution within the homogenisation time, and thus the ^{235}U might be high or low when there is an r-process excess.

For what it is worth, the difference in $^{207}\text{Pb}/^{206}\text{Pb}$ ratios between Angra dos Reis and the other two basaltic achondrites implies an order of magnitude $\Delta \sim 0.019/20 \sim 10^{-3}$. Since in this case it seems that ^{235}U is in excess in Angra dos Reis, it is perhaps not surprising that this meteorite also has a large component of the fission decay products of ^{244}Pu , an extinct radioactivity with a half-life of 82×10^6 yr (ref. 14).

Thus it seems that a case can be made that the isotopic differences between Angra dos Reis and other basaltic achondrites may be due to real fluctuations between the relative abundances of s-process and r-process nucleosynthesis products at a level $\Delta \sim 10^{-3}$. These differences are at a level which is subject to detection by careful mass spectrometry. The optimum ranges of elements in which to seek such effects are those in which there are comparable abundances from the two processes. Elements of even atomic number in the range from germanium to tellurium are probably most suitable for these measurements. If such differences should be found, this would provide a new tool for finding difference source regions in which Solar System materials have accumulated.

After this letter was submitted, I learned of the important work of R. N. Clayton, L. Grossman, and T. K. Mayeda (personal communication) showing that individual minerals in carbonaceous chondrites contain variations in oxygen isotope ratios that must represent different mixtures of separate nucleosynthetic processes. The general theoretical considerations given here should also provide an explanation of these effects.

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- ¹ Papanastassiou, D. A., and Wasserburg, G. J., *Earth planet. Sci. Lett.*, **5**, 361 (1969).
- ² Papanastassiou, D. A., thesis, CalTech (1970).
- ³ Tatsumoto, M., Knight, R. J., and Allegre, C. J., *Science*, N.Y., **180**, 1279 (1973).
- ⁴ Scalo, J. M., and Ulrich, R. K., *Astrophys. J.* (in the press).
- ⁵ Hoyle, F., and Fowler, W. A., *Nature*, **241**, 384 (1973).
- ⁶ Colgate, S. A., *Astrophys. J. Lett.*, **181**, 53 (1973).
- ⁷ Colgate, S. A., *Astrophys. J.* (in the press).
- ⁸ Reeves, H., *Astr. Astrophys.*, **19**, 215 (1972).
- ⁹ Spitzer, jun., L., *Diffuse Matter in Space* (Interscience, New York, 1968).
- ¹⁰ Spitzer, jun., L., in *Nebulae and Interstellar Matter* (edit. by Middlehurst, B. M., and Aller, L. H.) (University of Chicago Press, Chicago, 1968).
- ¹¹ Truran, J. W., and Cameron, A. G. W., *Astrophys. Space Sci.*, **14**, 179 (1971).
- ¹² Cameron, A. G. W., *Icarus*, **18**, 407 (1973).
- ¹³ Cameron, A. G. W., *Space Sci. Rev.* (in the press).
- ¹⁴ Hohenberg, C. M., *Geochim. cosmochim. Acta*, **34**, 185 (1970).

Post-depositional Remanent Magnetisation in Deep-sea Sediment

SEDIMENTS of various lithologies cored from most parts of the deep ocean floor have been found to contain a record of the past behaviour of the Earth's magnetic field, especially the sequence of reversals over at least the past 5 m.y. (ref. 1). The mechanism by which these sediments acquired their natural remanent magnetism (NRM) is, however, still poorly understood. We have recently conducted experiments that indicate that post-depositional remanent magnetisation² is a viable mechanism of magnetisation of deep-sea sediments.

Laboratory studies of the most obvious mechanism, depositional or detrital remanent magnetism (DRM), have shown that the remanent inclination is usually shallower than the field inclination in which the sediment was deposited^{3,4}. The difference is thought to be due to the effects of gravity overcoming magnetic-orienting couples of the magnetised grains at the instant of deposition⁵. The NRM inclinations of deep-sea sediments generally agree, however, with the expected axial dipole field inclinations⁶. It has been suggested⁷ that these sediments may acquire their remanent magnetism shortly after deposition, by the post-depositional mechanism observed in synthetic sediment². The physical origin of this remanence is likely to be closely related to Brownian motion⁸. In deep-sea sediments, a remanence may be acquired as a result of sediment disturbance by benthic organisms⁹. The natural remanent magnetism of some sediment may also be of chemical origin as a result of *in situ* authigenic growth of ferromagnetic minerals¹⁰.

Deep-sea sediment was obtained from the bottom of a Lamont core, RC14-14, the magnetic properties of which have been reported¹¹. The NRM was of a stable type; alternating fields necessary to randomise 50% of the original NRM (median destructive fields) averaged 200 oersted. The average magnetic inclination of the core was not significantly different from the axial dipole field inclination at the core site. The dominant magnetic minerals were essentially magnetite and titanomagnetite (compositional parameter, $x=0.53$) which were incorporated in a matrix of diatomaceous clay.

A quantity of the sediment was thoroughly dispersed in a sufficient amount of distilled water to form a thick slurry,

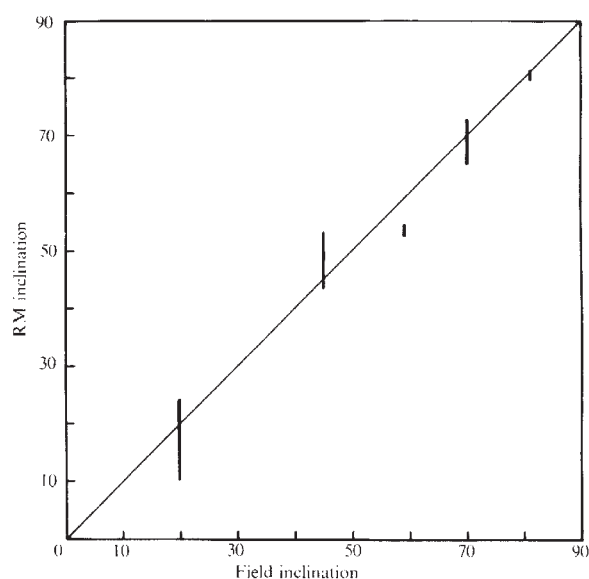


Fig. 1 Inclinations of the control field and the remanent magnetisation of the reconstituted sediment. Error bars represent range of remanent inclinations for each sample; the line of perfect agreement is shown. Successively steeper inclinations were produced in fields of 0.20, 0.28, 0.38, 0.59 and 1.20 oersted, respectively.

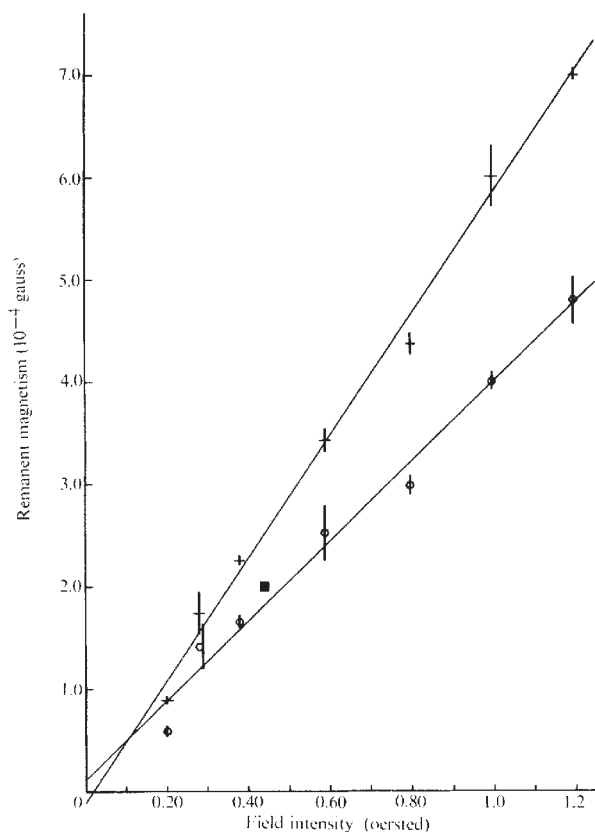


Fig. 2 Intensities of the control field and the remanences of the reconstituted sediment, before (+) and after (ϕ) 100 oersted a.f. demagnetisation. Regression line slopes are 5.96 ± 0.19 ($\times 10^{-4}$ gauss oersted $^{-1}$) and 3.87 ± 0.20 ($\times 10^{-4}$ gauss oersted $^{-1}$), respectively. ■, Average remanent intensity of RC14-14, after 100 oersted a.f. demagnetisation¹¹, plotted against the present axial dipole field intensity at the core site.

similar in consistency to that normally found near the tops of piston cores immediately after coring. The sediment slurry was spooned into plastic cylinders (diameter 7 cm, height 5 cm) that had perforated bottoms covered with paper towelling to prevent sediment loss. Helmholtz coils were used to provide various field intensities and directions. The sediment slurry was thoroughly stirred in the presence of a known field and allowed to dry in this same field. Two or three specimens were cored from each sample with a plastic cylinder after the sediment became cohesive yet not completely dry (generally after 4 to 7 d). The remanent magnetism of each specimen and that remaining after partial alternating field (a.f.) demagnetisation¹² were measured with a slow-spin magnetometer. Unreliable results were obtained if the sediment was too wet.

The remanent inclinations agreed reasonably well with the inclinations of the control fields (Fig. 1). No systematic deviation of the remanent magnetism direction from that of the control field, such as inclination error^{4,13}, was observed. The scatter of directions among specimens from an individual sample is attributed chiefly to specimen orientation errors and, more important, to visible deformation of the sediment resulting from partial desiccation.

The remanent intensity was linearly proportional to the intensity of the control field, at least up to 1.2 oersted (Fig. 2). The regression line through either the remanent intensities or the partially demagnetised remanences intersects the ordinate at a value not significantly different from zero magnetisation intensity. Only 35% of the remanence was randomised by 100 oersted a.f. demagnetisation as illustrated by the different slopes of the two lines. Alternating field demagnetisation curves of individual specimens show median destructive fields of about

200 oersted, with corresponding directional changes of less than 10° .

The process of stirring the sediment in the presence of a magnetic field may be analogous to the activities of benthic organisms at, or just beneath, the sediment-water interface on the ocean floor¹⁴. Core RC14-14 shows evidence of benthic organism activity or bioturbation over most of its length¹¹. The NRM of the core and the remanence of the reconstituted sediment are similar in intensity (Fig. 2), in stability against alternating fields and in their apparent lack of systematic inclination error. This evidence suggests that the stable NRM is a post-depositional remanent magnetisation acquired in conjunction with physical disturbance by benthic organisms in most sections of the core.

Mixing of deep-sea sediment by benthic organisms is seen from photographs to be a common feature of the ocean bottom environment¹⁵ and evidence of it is found in many sediment cores^{16,17}, which often contain good magnetic records^{18,19}. Therefore, a substantial portion of deep-sea sediment may have acquired an NRM as a result of a sediment-mixing process. This is not to say that mixing is a prerequisite for the acquisition of post-depositional remanence in sediment. Preliminary results show that the same sediment acquires a stable remanence in the direction of an applied field without stirring. It has no inclination error but has a somewhat lower intensity. Even if the DRM mechanism is also a factor in the original magnetisation of deep-sea sediment, realignment of this type of remanence may occur after deposition. The small grain diameters of magnetic minerals often encountered in deep-sea sediment (generally $40\% < 1 \mu\text{m}$)²⁰, coupled with the field dependence and lack of inclination error of this post-depositional remanence, support the Brownian motion model of magnetic grain alignment in a field in the sediment^{8,21}.

The extent to which the sediment and its observed remanent magnetism are contemporaneous must vary with depth of faunal activity and other factors controlling the immobilisation of magnetic grains in the sediment. These include grain size, grain magnetic moment and sediment-water content. The mean burrowing depths are apparently only about 5 cm in deep-sea sediment, although some burrows can infrequently extend deeper^{17,22,23}. Preliminary laboratory observations indicate that only a small decrease in water content is necessary to lock-in a post-depositional remanence in the sediment. This suggests that the difference in depth between acquisition of remanence and the sedimentation surface may not be excessive.

The applicability of such sediment reconstitution studies to palaeofield intensity measurements in deep-sea sediments is now being investigated.

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¹ Opdyke, N. D., *Rev. Geophys. Space Phys.*, **10**, 213 (1972).

² Irving E. and Major, A., *Sedimentology*, **3**, 135 (1964).

³ Johnson, E. A., Murphy, T., and Torreson, O. W., *Terr. Magn. Atmos. Elect.*, **53**, 349 (1948).

⁴ King, R. F., *Mon. Not. R. astr. Soc. Geophys. Suppl.*, **7**, 115 (1955).

⁵ King, R. F., and Rees, A. I., *J. geophys. Res.*, **71**, 561 (1966).

⁶ Opdyke, N. D., and Henry, K. W., *Earth planet. Sci. Lett.*, **6**, 139 (1969).

⁷ Kobayashi, K., Kitazawa, K., Kanaya, T., and Sakai, T., *Deep-Sea Res.*, **18**, 1045 (1971).

⁸ Collinson, D. W., *J. geophys. Res.*, **70**, 4663 (1965).

⁹ Watkins, N. D., *Earth planet. Sci. Lett.*, **4**, 341 (1968).

¹⁰ Harrison, C. G. A., and Peterson, M. N. A., *Am. Miner.*, **50**, 704 (1965).

¹¹ Opdyke, N. D., Kent, D. V., and Lowrie, W., *Earth planet. Sci. Lett.* (in the press).

- ¹² McElhinny, M. W., *Geophys. J. R. astr. Soc.*, **10**, 369 (1966).
¹³ Griffiths, D. H., King, R. F., Rees, A. I., and Wright, A. E., *Proc. R. Soc.*, **A256**, 359 (1960).
¹⁴ Harrison, C. G. A., *J. geophys. Res.*, **71**, 3033 (1966).
¹⁵ Laughton, A. S., in *The Sea* (edit. by Hill, M. N.), **3**, 437 (Interscience, 1963).
¹⁶ Glass, B. P., *Earth planet. Sci. Lett.*, **6**, 409 (1969).
¹⁷ Ruddiman, W. F., and Glover, L. K., *Geol. Soc. Am. Bull.*, **83**, 2817 (1972).
¹⁸ Hays, J. D., Saito, T., Opdyke, N. D., and Burckle, L. H., *Geol. Soc. Am. Bull.*, **80**, 1481 (1969).
¹⁹ Foster, J. H., and Opdyke, N., *J. geophys. Res.*, **75**, 4465 (1970).
²⁰ Løvlie, R., Lowrie, W., and Jacobs, M., *Earth planet. Sci. Lett.*, **15**, 157 (1971).
²¹ Stacey, F. D., *Pure Appl. Geophys.*, **98**, 139 (1972).
²² Ericson, D. B., Ewing, M., Wollin, G., and Heezen, B. C., *Geol. Soc. Am. Bull.*, **72**, 193 (1961).
²³ Griggs, G. B., Carey, jun., A. G., and Kulm, L. D., *Deep-Sea Res.*, **16**, 157 (1969).

Flocculation of Latices by Low Molecular Weight Polymers

POLYELECTROLYTES are increasingly being used as flocculants for electrostatically stabilised dispersions of colloidal particles in such diverse fields as water treatment and mineral processing¹. Suitable polyelectrolytes, which are usually effective at low concentrations (≤ 10 p.p.m.), invariably have a high molecular weight² (a viscosity average molecular weight $M_v > 10^6$). They seem to function by a bridging mechanism whereby the flocculant interferes with the free movement of the particles¹⁻⁶.

We now report that even low molecular weight non-ionic polymer molecules ($M_v \geq 10^3$ to 10^4) are able to flocculate some polymer latices. They also seem to function by a bridging mechanism but require the presence at the particle surfaces of suitable groups with which the flocculant can interact specifically.

The new phenomenon was first discovered with an apparently electrostatically stabilised aqueous polystyrene latex (mean radius 24 nm), prepared by the method of Ottewill and Shaw⁷. Figure 1a displays the course of flocculation, which was followed turbidimetrically, on addition of poly(ethylene oxide) (PEO) to the latex at 35°C. (The initial light scattering was compensated for.) Several samples of low molecular weight PEO (ex Union Carbide Australia Ltd) were examined using the latex at a particle concentration of $\sim 10^{12}$ cm⁻³. In the absence of PEO the particles exhibited long term stability, whether or not 0.2 M HCl was present (line 1). When low molecular weight PEO was added in the absence of 0.2 M HCl no flocculation could be detected at any polymer concentration. But the addition of PEO, at 20 p.p.m., of either $M_v = 4,000$ or 23,000 in the presence of 0.2 M HCl resulted in rapid flocculation of the latex (curves 2 and 3). The higher molecular weight polymer was the more effective. Very high molecular weight polymer $M_v = 1 \times 10^6$ was also an effective flocculant at concentrations of only a few p.p.m.

Polystyrene latices prepared by the method of Ottewill and Shaw⁷ possess surface carboxylic acid groups^{8,9}. To investigate the role of these groups, polystyrene latices were prepared at low temperatures ($< 30^\circ$ C); as we have shown elsewhere¹⁰, such latices possess no detectable surface carboxylic acid groups. Interestingly these latices failed to flocculate on addition of PEO in the presence of 0.2 M HCl. On the other hand, polystyrene latices sterically stabilised¹¹ by poly(acrylic acid) (PAA) of $M_v = 19,300$ were also found to be flocculated by low (and high) molecular weight PEO (Fig. 1b). Again 0.2 M HCl was necessary for flocculation. We note that in the absence of PEO these latices exhibit long term entropic stabilisation¹¹ (line 4).

The foregoing experiments point to the central role played

by the surface carboxylic acid group in promoting flocculation. They suggest the following mechanism for flocculation by low molecular weight polymers: At suitably low pH the surface carboxylic acid groups are predominantly unionised and so can H bond with the ether oxygens of the PEO chains. The occurrence of such H bonding is forcefully demonstrated by the phase separation of mixtures of PAA and PEO from aqueous solutions at low pH (refs 12 and 13). For the conditions relevant to curve 3 in Fig. 1a, the centres of the particles were separated, on average, by $\sim 1,000$ nm, if a cubic array is assumed. The r.m.s. end-to-end length of the PEO chains was calculated¹⁴ to be only ~ 10 nm. Obviously on average the polymer chains could not possibly span the distance between the particle surfaces. Therefore the PEO chains may reasonably be inferred to have become attached to one particle by H bonding to the surface carboxylic acid groups and to have become attached to a second (or more) particle(s) in subsequent Brownian collisions. Free movement of the latex particles was thus prevented, corresponding to bridging flocculation. According to this postulated mechanism, no flocculation would be expected at higher pH values because the ionised carboxylic acid groups would be unable to H bond with the PEO. Also no flocculation would be expected in the absence of surface carboxylic acid groups. Both expectations were realised.

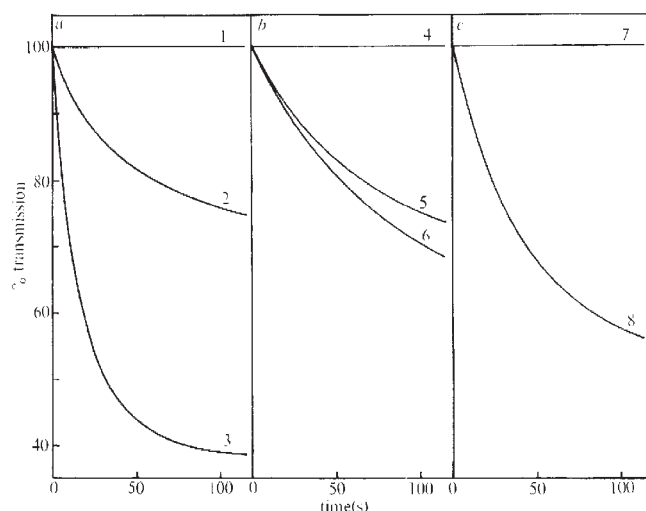


Fig. 1 The % transmission as a function of time for polystyrene latices. *a*, Curves for latices containing surface carboxylic acid groups: 1, no PEO added; 2, PEO of $M_v = 4,000$ added; 3, PEO of $M_v = 23,000$ added. *b*, Curves for latices stabilised by PAA: 4, no PEO added; 5, PEO of $M_v = 4,000$ added; 6, PEO of $M_v = 23,000$ added. *c*, Curves for latices stabilised by PEO: 7, no PAA added; 8, PAA of $M_v = 19,300$ added. Flocculant added at 20 p.p.m. in *a* and *c* and 50 p.p.m. in *b*.

If this explanation is correct then it is also predicted that low molecular weight PAA should be able to flocculate latices stabilised solely by PEO chains. Figure 1c shows the flocculation induced by the addition of PAA ($M_v = 19,300$) to a polystyrene latex sterically stabilised by PEO ($M_v = 23,000$). Again no flocculation was evident unless 0.1 M HCl was also present to convert most of the carboxylic acid groups into the unionised form.

The phenomenon seems to be closely analogous to the bridging flocculation observed with high molecular weight polyelectrolytes. Both are characterised by the intervention of the polymeric flocculant by promoting 'sticky' collisions between the particles, the flocculated particles often being separated by some distance¹⁵. There is thus no need to reduce to near zero the repulsive potential energy between

the particles, whether electrostatically or sterically generated, to permit flocculation to occur.

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- ¹ Napper, D. H., and Hunter, R. J., *M.T.P. Int. Rev. Sci., phys. Chem. Ser.*, **1**, 7, 294 (1972).
- ² La Mer, V. K., and Healy, T. W., *Rev. pure appl. Chem.*, **13**, 112 (1963).
- ³ Ries, jun., H. E., and Meyers, B. L., *Science, N.Y.*, **160**, 1449 (1968).
- ⁴ Gregory, J., *Trans. Faraday Soc.*, **65**, 2260 (1969).
- ⁵ Ries, jun., H. E., *Nature*, **226**, 72 (1970).
- ⁶ Williams, D. J. A., and Ottewill, R. H., *Kolloid-Z. Z. Polym.*, **243**, 141 (1971).
- ⁷ Ottewill, R. H., and Shaw, J. N., *Kolloid-Z. Z. Polym.*, **215**, 161 (1967).
- ⁸ Ottewill, R. H., and Shaw, J. N., *Kolloid-Z. Z. Polym.*, **218**, 34 (1967).
- ⁹ Shaw, J. N., and Marshall, M. C., *J. Polym. Sci. Pt A-1*, **6**, 449 (1968).
- ¹⁰ Smitham, J. B., Gibson, D. V., and Napper, D. H., *J. Colloid Interface Sci.* (in the press).
- ¹¹ Evans, R., Davison, J. B., and Napper, D. H., *J. Polym. Sci., Pt. B*, **10**, 449 (1972).
- ¹² Bailey, F. E., Lundberg, R. D., and Callard, R. W., *J. Polym. Sci. Pt. A*, **2**, 845 (1964).
- ¹³ Bailey, F. E., and Koleske, J. V., *Nonionic Surfactants* (edit. by Schick, M. J.), **1**, 819 (Dekker, New York, 1967).
- ¹⁴ Kurata, M., Iwama, M., and Kamada, K., *Polymer Handbook* (edit. by Brandrup, J., and Immergut, E. H.), IV-58 (Interscience, New York, 1966).
- ¹⁵ Wallis, W. E., *J. Colloid Interface Sci.*, **27**, 77 (1968).

BIOLOGICAL SCIENCES

Genetic Differences in Conformational Changes of Albumin in the House Mouse

THE ability of mouse liver to convert mouse or bovine albumin to a faster migrating band under acid conditions of electrophoresis has recently been found to be controlled by a dominant gene, as observed in the SWR/J and several other strains of mice¹. Other strains, such as C3H/HeJ, had the recessive allele and were unable to convert albumin. The conditions used included a pH at which conformational changes in albumin are known to occur, suggesting that the strains of mice differ in a factor in the liver capable of converting albumin to a different conformational state. I report here evidence that the conversion of albumin can be reversed by lowering the pH markedly. This provides further evidence that a conformational change has taken place.

Livers from male C3H/HeJ and SWR/J mice were homogenized and centrifuged, and the supernatant was extracted using procedures described before¹. One volume of horse serum albumin (HSA, Fraction V, Sigma, 5 mg ml⁻¹ in 0.25 M sucrose) was added to 9 volumes of extract. An equal volume of solvent (citric acid, monohydrate: 15% sucrose, 1:5 (w/v), plus 6 M urea) was added to each extracted sample. Before electrophoresis 10 μ l was added to a slot, 5 volumes of an aliquot was diluted with 1 volume of concentrated HCl and 20 min later 12 μ l was added to another slot, and finally 10 μ l of an aliquot untreated with HCl was added to a third slot. Controls were also included in which 0.25 M sucrose replaced HSA or liver supernatant, but were otherwise handled as other

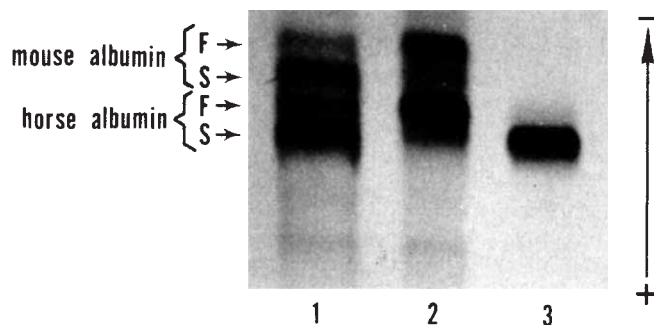


Fig. 1 Electrophoretograms showing the effect of liver extract and HCl on HSA. Channels 1-3 are (1) HSA+SWR/J liver extract+HCl; (2) HSA+SWR/J liver extract, and (3) HSA. Electrophoresis was as previously described¹. Gel buffer was 0.05 M Tris-citric acid, pH 2.4+3 M urea+10% 'Cyanogum'-41. Electrode buffer was 0.2 M glycine-citric acid, pH 3.5.

samples, although not treated with HCl. HSA was used because both slow and fast forms were electrophoretically distinct from proteins of mouse liver.

Results are shown in Fig. 1. HSA was present as a single, slower migrating band when not combined with liver (channel 3), whereas most was converted to a faster band when combined with liver extract of the SWR/J strain (channel 2). The latter result was not affected by the time when the sample was added to the slot, that is, before addition of HCl to an aliquot more than 20 min before electrophoresis compared with just before electrophoresis. Treatment with HCl (channel 1) lowered pH markedly (from 2.83 to 0.05) and converted much of the HSA to the slower migrating band. In C3H/HeJ mice (results not shown) HSA was not converted to a faster migrating band, but consisted only of a slow band migrating the same distance as in channel 3, with or without added HCl. Most of the mouse albumin of liver was also converted to a faster migrating band in the SWR/J but not the C3H/HeJ strain, as described previously¹; treatment with HCl converted a large portion to the slower migrating band in the SWR/J strain (Fig. 1), but there was no change in the C3H/HeJ strain.

My data show that liver of one strain of mouse but not of another can convert HSA to a faster migrating form. The genetic difference is clearly not in the primary structure of albumin, that is, amino acid sequence, since the same source of HSA was used in all preparations, but is rather a secondary alteration of the molecule. A conversion to a different conformational state is highly likely in view of the reversal from the faster to the slower migrating band after treatment with excess acid, especially since lowering the pH below 3.5 favours the expanded, slower migrating form². The reversibility of the change in albumin renders untenable the hypothesis that conversion of albumin from a slower to faster migrating form results from cleavage of a portion of the albumin molecule. These results point to the existence of two genes which control the structure of albumin, that is a conventional "structural gene" specifying primary structure³ and a second gene affecting secondary or tertiary structure.

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¹ Wilcox, F. H., *Biochem. Genet.*, **10**, 69 (1973).

² Sogami, M., and Foster, J. F., *J. biol. Chem.*, **237**, 2514 (1962).

³ Petras, M. L., *Biochem. Genet.*, **7**, 273 (1972).

Platelet Contractile Regulation in an Isometric System

WE have previously shown that human platelets, in addition to actomyosin, the so-called thrombosthenin¹, contain a tropomyosin-like protein². Furthermore, we provided evidence for the presence in isolated platelet actomyosin of a control system which "turns-on" the Mg-ATPase of the contractile protein complex at a saturating level of 10^{-6} M Ca^{2+} (ref. 2).

Platelet contraction has usually been considered to be an irreversible process. Platelets have been found, however, to contain a membrane-associated relaxing factor, resembling the muscle sarcotubular system. This factor sequesters calcium from solution and behaves as a Ca^{2+} pump^{3,4}. It has been shown recently that in an isotonic contractile system, a platelet-containing clot relaxes in the presence of papaverine, theophylline or cytochalasin B (ref. 5). Here we used an isometric system to measure platelet contraction and relaxation and the calcium-sensitivity of these processes.

Cylindrical clots were obtained by pouring a human platelet-rich plasma suspension, immediately after thrombin addition, into siliconized glass tubes, 6 × 0.6 cm internal diameter, plugged at one end with parafilm. Saline was carefully layered over the suspension to abolish the meniscus. After 10 min the clot cylinder was poured into a Petri dish containing ice cold Tyrode solution to inhibit contraction. The clot was then tied at one end with a cotton thread to a rigid glass support and at the other end to a Grass force displacement transducer (FT 03 C) linked to a Grass Polygraph d.c. driver amplifier recording system. The clot was immersed in a bath of Tyrode solution containing 1.7 mM Ca^{2+} at 37° C and the tension was recorded.

The tension developed in the clot in this Ca^{2+} -containing isometric system increased with rising platelet concentration, similar to observations on clot retraction measured in a contractile isotonic system^{6,7}. The maximal tension was proportional to the initial cross-sectional area of the clot, but was independent of its length. Unlike in a muscle system, the cross-sectional area of the clot greatly decreased during isometric contraction, presumably as a result of transverse contraction associated with extrusion of serum from the fibrin-platelet network. In the Ca^{2+} -containing isometric system (experimental conditions described in the legend to Fig. 1), platelet-rich clots developed tension at an initial speed of 0.2 mg min⁻¹ cm⁻² (initial cross-sectional area) and reached a maximal tension of 1.8 mg cm⁻² after 15 min. After maintaining a tension plateau for about 5 min, the clot relaxed spontaneously and irreversibly at a rate approximately half that of the development of the tension. This spontaneous irreversible

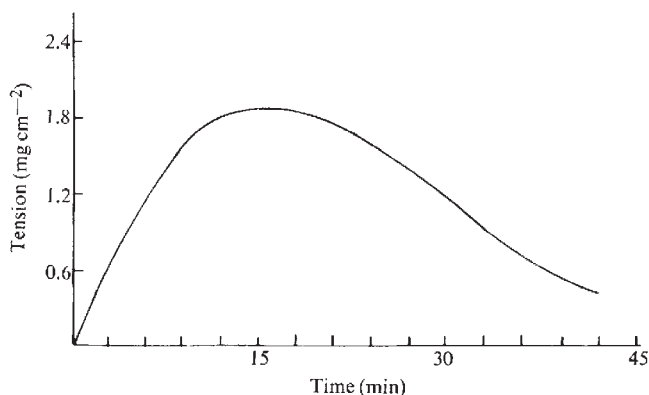


Fig. 1 Tension development in thrombin-clotted platelet-rich plasma in a Ca^{2+} -containing isometric system. Citrated platelet-rich plasma, 2 ml, containing 300,000 platelets mm⁻³ and 250 mg% fibrinogen, were clotted with 0.5 NIH units thrombin (Parke-Davis). The clot was incubated at 37° C in Tyrode solution, containing 1.7 mM Ca^{2+} . The tension recorded is expressed in mg cm⁻² (initial cross-section area).

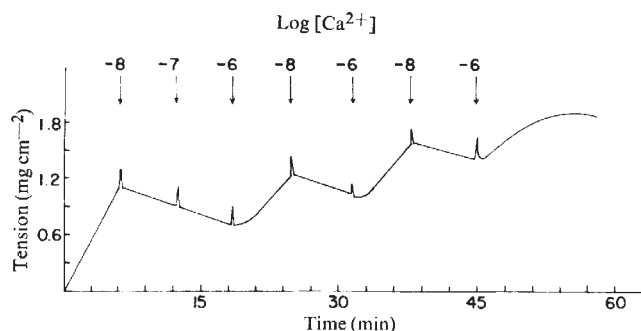


Fig. 2 Effect of Ca^{2+} on the tension developed in a thrombin-clotted platelet-rich plasma. Except for Ca^{2+} concentration, the conditions were the same as in Fig. 1. The clot was washed and incubated in Tyrode solution containing variable Ca^{2+} concentrations in the pre-maximal contraction phase. Appropriate mixtures of 10 mM EGTA and 10 mM Ca-EGTA were used to obtain the desired Ca^{2+} concentrations, taking into account a dissociation constant of 1.9×10^{-7} for Ca-EGTA (ref. 29).

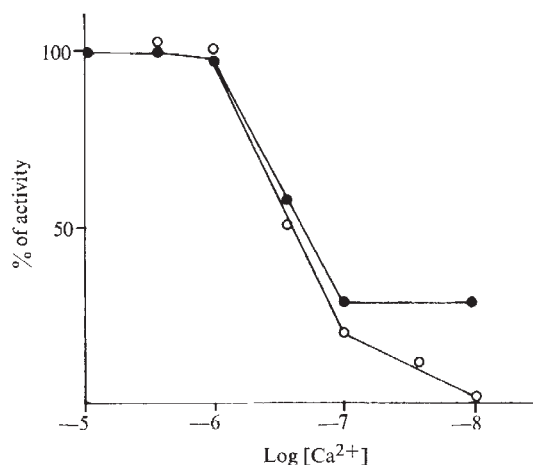


Fig. 3 Effect of Ca^{2+} on isotonic clot retraction and on Mg-ATPase activity of human platelet actomyosin. O, Citrated platelet-rich plasma was clotted with 0.5 NIH units ml⁻¹ of thrombin in flamed test tubes in the presence of 10^{-8} M to 10^{-5} M Ca^{2+} adjusted with Ca-EGTA buffer as in Fig. 2. The percentage of clot retraction was calculated from the measured volume of expressed serum, 100% being defined as maximal clot retraction. ●, Mg-ATPase activity of human platelet actomyosin at varying Ca^{2+} concentrations was estimated as described elsewhere².

relaxation may be a sequel to platelet ATP depletion known to occur as a result of thrombin action^{8,9} and possibly to platelet membrane damage inherent in viscous metamorphosis¹⁰.

When a contracting clot, at pre-maximal tension, was treated with Ca^{2+} -free Tyrode solution containing 0.05 M ethylene glycol-bis-(2-aminoethyl ether) N',N'-tetraacetic acid (EGTA, a selective Ca^{2+} -chelator), relaxation immediately occurred (Fig. 2). When subsequently bathed again in Ca^{2+} -containing Tyrode, the clot contracted at the original initial speed. Addition of EGTA at the time when maximal tension was reached or later did not affect the speed of the spontaneous relaxation. When a contracting clot had developed half its maximal tension, it could be relaxed by lowering the free Ca^{2+} concentration (by the use of an EGTA-Ca buffer) to 10^{-8} M. The minimal concentration of Ca^{2+} required for maximal contraction was found to be 10^{-6} M, similar to muscle and platelet natural actomyosin systems². Cycles of contraction and relaxation could be induced several times in the same clot by varying the Ca^{2+} concentration (Fig. 2).

The effect of Ca^{2+} in a standard isotonic clot retraction system was studied for comparison. Retraction of a thrombin-treated platelet-rich plasma clot was half maximum at 3×10^{-7} M Ca^{2+} and reached maximum value above 10^{-6} M Ca^{2+} (Fig. 3). The latter Ca^{2+} concentration was identical

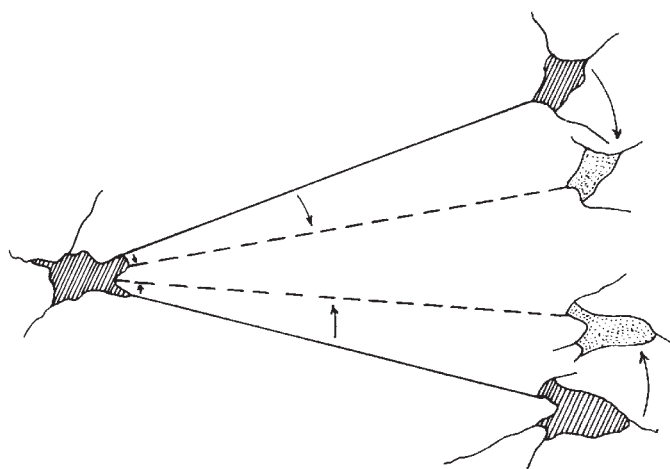


Fig. 4 Clot concentration model. Platelet masses connected by fibrin fibres. The contraction of the platelet masses results in a reduction of the angle between the fibrin fibres. —, Fibrin fibre before contraction; ---, fibrin fibre after contraction.

to both that required for maximal ATPase activity of thrombosthenin and that required for maximal tension of the platelet-rich plasma clot in the isometric system described here.

This study has shown that the clot contraction-relaxation cycle depends upon the presence of Ca^{2+} . Calcium, particularly intracellular platelet calcium, has long been suspected to be an essential requirement for clot contraction⁷. Indeed, platelets have been shown to contain high concentrations of Ca^{2+} which could be released by thrombin¹¹. The presumably intracellularly-bound Ca^{2+} would, in the process of release, be expected to interact with specific platelet actomyosin sites^{12,13}; subsequent interaction between actin and myosin would then occur and contraction would follow. In our study, thrombin-treated platelets showed permeability properties similar to glycerinated or skinned muscle fibres rather than intact muscle fibres, as they responded to externally added EGTA.

Two mechanisms may be suggested to account for the dependence of platelet contraction on the exterior concentration of EGTA-Ca^{2+} : the action of Ca^{2+} on thrombosthenin located on the outer surface of the platelet membrane, or the occurrence of a selective change in the platelet membrane permeability. In support of the first mechanism several authors have found thrombosthenin and thrombosthenin-specific ecto-ATPase on the external surface of the platelet membrane¹⁴⁻¹⁷, but these studies are considered inconclusive¹⁸. In support of the second mechanism it may be mentioned that thrombin is able to hydrolyse platelet membrane proteins¹⁹⁻²¹. Consequently, a change of platelet membrane protein conformation might occur and affect the local lipid structure, as has been discussed by Singer^{22,23} for hypothetical models. In addition, extracellular calcium can bind to phospholipid heads of synthetic lipid bilayers, neutralize their charges and produce a compression wave which is effective at long distances²³⁻²⁵. Such Ca^{2+} -generated compression waves might cause structural changes in proteins embedded in these lipid bilayers²³. This effect may explain the need for extracellular Ca^{2+} for platelet aggregation and contractile processes. Furthermore, thrombin has been shown to increase the rate of Ca^{2+} uptake by platelets¹¹.

The generation of tension in a contracting clot involves two processes, platelet contraction itself, and fibrin network collapse with extrusion of trapped serum. The mechanism by which platelets pull the fibrin fibres together is poorly understood. We venture to propose a model (I. C., and E. Katchalski, unpublished) taking into account the rigidity of the fibrin fibres which during clotting become intimately associated with the platelet membranes and converge towards the platelet

masses^{26,27}. Contraction of the platelet mass would reduce the angle between the attached fibrin fibres, their approximation amplifying with distance. Other platelet masses associated with these fibrin fibres would approach each other and tension would be generated in the clot (Fig. 4).

The clot contraction-relaxation cycles demonstrated in our experiments stress the physiological significance in platelets of both the sarcotubular system, functioning as a Ca^{2+} pump, and the contractile protein regulatory system. Our previous observations²⁸ support a regulation of the platelet contractile process of an actin-linked tropomyosin-troponin type rather than of a myosin-linked type.

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- ¹ Bettex-Galland, M., and Lüscher, E. F., *Biochim. biophys. Acta*, **49**, 536 (1961).
- ² Cohen, I., and Cohen, C., *J. molec. Biol.*, **68**, 383 (1972).
- ³ Grette, K., *Nature*, **198**, 488 (1963).
- ⁴ Statland, B. E., Heagan, B. M., and White, J. G., *Nature*, **223**, 521 (1969).
- ⁵ Majno, G., Bouvier, C. A., Gabbiani, G., Ryan, G. B., and Statkov, P., *Thromb. Diath. Haemorrh.*, **28**, 49 (1972).
- ⁶ Budtz-Olsen, O. E., in *Clot Retraction* (Blackwell Scientific Publications, Oxford, 1951).
- ⁷ Lüscher, E. F., *Vox Sang.*, **1**, 133 (1956).
- ⁸ Ireland, D. M., *Biochem. J.*, **105**, 857 (1967).
- ⁹ Holmsen, H., and Day, H. J., *J. Lab. clin. Med.*, **75**, 841 (1970).
- ¹⁰ Rodman, N. F., and Mason, R. G., *Fedn. Proc.*, **26**, 95 (1967).
- ¹¹ Mürer, E. H., and Holme, R., *Biochim. biophys. Acta*, **222**, 197 (1970).
- ¹² White, J. G., *Blood*, **31**, 604 (1968).
- ¹³ Day, H. J., and Holmsen, H., *Ser. Haematol.*, **4**, 3 (1971).
- ¹⁴ Booyse, F. M., and Rafelson, jun., M. E., *Ser. Haematol.*, **4**, 152 (1971).
- ¹⁵ Salzman, E. W., Chambers, D. A., and Neri, L. L., *Nature*, **210**, 167 (1966).
- ¹⁶ Chambers, D. A., Salzman, E. W., and Neri, L. L., *Archs. Biochem. Biophys.*, **119**, 173 (1967).
- ¹⁷ Rossi, E. S., *Blood*, **34**, 543 (1969).
- ¹⁸ Lüscher, E. F., Probst, E., and Bettex-Galland, M., *Ann. N.Y. Acad. Sci.*, **201**, 122 (1972).
- ¹⁹ Cohen, I., Bohak, Z., de Vries, A., and Katchalski, E., *Eur. J. Biochem.*, **10**, 388 (1969).
- ²⁰ Baenziger, N. L., Brodie, G. N., and Majerus, P. W., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 240 (1971).
- ²¹ Jamieson, G. A., Fuller, N. A., Barber, A. J., and Lombart, C., *Ser. Haematol.*, **4**, 125 (1971).
- ²² Singer, S. J., *Ann. N.Y. Acad. Sci.*, **195**, 16 (1972).
- ²³ Singer, S. J., in *Structure and Function of Biological Membranes* (edit. by Rothfield, L. I.), 145 (Academic Press, New York, 1971).
- ²⁴ Rubalcava, B., Martinez de Munoz, D., and Gitler, C., *Biochemistry, N.Y.*, **8**, 2742 (1969).
- ²⁵ Vanderkooi, J., and Martonosi, A., *Archs. Biochem. Biophys.*, **133**, 153 (1969).
- ²⁶ White, J. G., Krivit, W., and Vernier, R. L., *Blood*, **25**, 241 (1965).
- ²⁷ Johnson, S. A., Fredell, L., Shepard, J. A., Tebo, T. H., Chang, C., Pederson, H. J., and Van Horn, D. L., in *Physiology of Hemostasis and Thrombosis* (edit. by Johnson, S. A., and Secgers, W. H.), 44 (Thomas, Springfield, Ill., 1967).
- ²⁸ Cohen, I., Kaminski, E., and de Vries, A., *FEBS Lett.* (in the press).
- ²⁹ Chaberek, S., and Martell, A. E., *Organic Sequestering Agents* (Wiley, New York, 1959).

Does Gonadotrophin-induced Ovulation in Mice cause Malformations in the Offspring?

GONADOTROPHIN treatment affects follicular maturation and induces ovulation in immature and mature mammalian females. This procedure has been used for experimental

research¹⁻⁸ as well as for clinical treatment in human anovulatory disease. Side effects reported when hormonally induced ovulation is followed by pregnancy include: frequent resorptions, spontaneous abortions with chromosomal anomalies, extrauterine and multiple pregnancies and difficulties in giving birth⁹⁻¹⁶.

This article demonstrates that after hormonally induced ovulation, malformations can occur in the embryos of mice. This is of interest, as Dyson and Kohler reported two clinical cases of anencephaly following gonadotrophin-induced ovulation¹⁷.

Ninety randomly bred virgin mice (Swiss albino), 6 to 9 weeks old, were caused to ovulate by intraperitoneal injections of 5 IU pregnant mare's serum gonadotrophin (PMS, 'Gestyl', Organon, Holland) in 0.15 ml of 0.9% saline and of 5 IU human chorionic gonadotrophin (HCG, 'Pregnyl', Organon, Holland) in 0.1 ml of 0.9% saline 48 h later. After the second injection the females were caged overnight with males (same strain and of proven fertility). Insemination was verified the following morning by the presence of a copulatory plug.

Table 1 Observations on Pregnant Females Following Gonadotrophin Treatment and Normal Mating

	Number	Injected (%)
Treated	90	
Mated	36	38.9
Pregnant	32	34.4
		Pregnant (%)
Deaths*	2	6.5
Normal delivery†	2	6.5
Complicated delivery‡	2	6.5
Blocked delivery§	26	80.6

* Death after hormone treatment occurred on the 18th d of gestation. These females had three and twelve normal foetuses respectively.

† One female gave birth to thirteen normal embryos, the other delivered three living and eight dead embryos.

‡ Females which had difficulties during delivery started to give birth to both dead and living embryos. They also failed to give complete birth, feeding on the embryos delivered first. They were autopsied 6 h after they had started to deliver; the normal length of the delivery procedure does not exceed 2 h. Living foetuses were still found in the uterus.

§ Untreated control females delivered between the 19th and 20th d of gestation, so animals which had shown blood-stained vaginal smears for 2 d were killed between the 19th and 21st d, the uteri were opened and respiration was induced manually in the individual embryos. Those which survived more than 1 h were placed with foster mothers, which had delivered on the same day and had been deprived of their litters.

Table 2 Reproductive Performance of Gonadotrophin-treated Females

	Treated	Control
Litters	32	8
Litters with anomalies	5	0 0.05 > P > 0.01
Average litter size	14.3 ± 6.5	12.4 ± 2.7*
No. of nidations	665	112
Living offspring	48.5%	93.8%
Dead foetuses	20.3%	1.8%
Anomalous (total offspring)	1.8%	0 0.01 > P > 0.001
Resorbed in utero	31.2%†	4.5%
No. of sexed embryos	336	521‡
Males	35.5%	51%
Females	64.6%	49% P < 0.001
Embryos placed to foster mothers	184	84
Viability up to 1 h	76%	99%
Survival to 23 d	66%	85%

* In addition to the controls (Caesarean sectioned), twenty-four normally delivering females of the same strain were used to estimate average litter size.

† Partly with malformations.

‡ For the estimation of the sex ratio in controls, an additional thirty-six normally delivering females of the same strain were used.

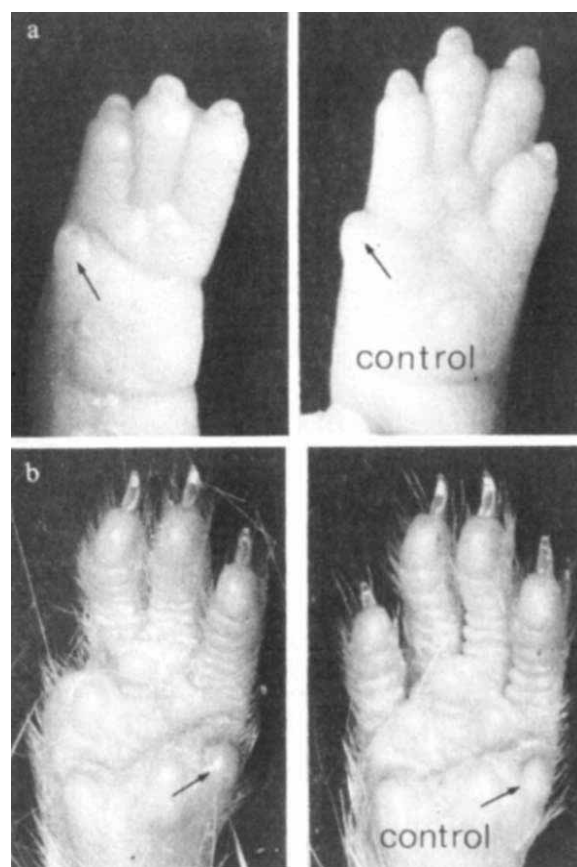


Fig. 1 Malformations of the forepaw (aplasia of the 5th digit, thumb arrowed). *a*, Left forepaw of newborn mice; *b*, right forepaw of mice 23 d post partum (the offspring of the treated female died at this age).

This day was designated as day 1 of pregnancy. No further treatment was given.

The results obtained with gonadotrophin-treated and normally mated mice are given in Table 1. Details of pregnancies at the time of autopsy of females subjected to gonadotrophin treatment are given in Table 2. The litters of females injected with hormones ranged in size from two to twenty-six, control litter size was between nine and sixteen. Although 99% of a total of eighty-four control embryos could be placed to foster mothers, only 76% of the embryos (total 184) obtained from treated females survived the first hour. Most of the embryos of injected females died within the first 5 d and those which lived to 23 d post partum survived thereafter. Death of controls occurred only during the first 3 d. This corresponds with the usual death rate of normally bred animals.

The viability of embryos of treated females of approximately the same litter size as of controls (up to sixteen embryos) is 64.4% (compared with 85% of controls), whereas the rate of survival in embryos of all treated females (including high litters also) is 66%. This indicates that embryonic death is not a result solely of excessive litter size in treated females.

Embryos of females injected with hormone, as well as of controls, were sexed after artificial and normal birth respectively. The sex ratio of males to females is 1 : 1.82 in the treated animals, whereas the controls show the usual slight imbalance in favour of males (ratio 1 : 0.96). This suggests that gonadotrophin treatment before pregnancy directly influences the distribution between the sexes.

The observed malformations are illustrated in Figs 1 and 2. Embryos of females (hormone-injected) with a litter size between fifteen and twenty-two showed postaxial oligodactylism of the forelimb. One embryo had a forelimb dysmelia of the reduction type which was also seen on late resorptions. Seven

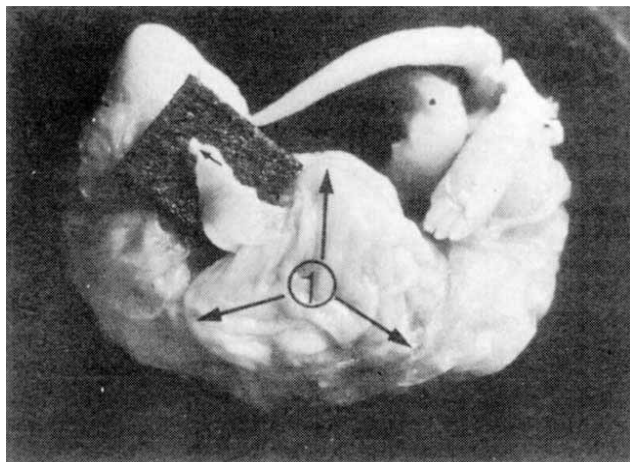


Fig. 2 Malformation of the right forepaw (dysmelia) and cystic bladder ①. This animal was dead at the time of Caesarean section. Note the absence of digits 2, 3, 4 and 5. The thumb (arrow) is suppressed to a thin structure.

of eight embryos were found to be abnormal with respect to the right forelimb (six females, one male), whereas the eighth (male) showed an aplasia of the left forelimb. The abnormalities observed in these experiments have a striking similarity to less severe lesions obtained in rats treated with acetazolamide during mid-pregnancy^{18,19} and in mice treated with 5-fluoro-2-deoxycytidine²⁰. The malformations reported here are phenocopies of the oligodactylism described by Grüneberg²¹ where, as a rule, the postaxial areas of the limb skeleton are also affected. The only difference is that in oligodactylism the left forelimbs are slightly more often affected, whereas in the observations reported here, as after treatment with acetazolamide, more right forelimbs were affected.

This study has shown that gonadotrophin treatment before pregnancy can result in statistically significant anomalies and a shifted sex ratio, as well as in a reduced survival chance after Caesarean section.

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- ¹ Rowlands, I. W., *J. Endocr.*, **3**, 384 (1944).
- ² Runner, M. N., and Palm, J., *J. expl. Zool.*, **124**, 303 (1953).
- ³ Lamond, D. R., *J. Endocr.*, **20**, 277 (1960).
- ⁴ Wilson, E. D., and Zarrow, M. X., *J. Reprod. Fert.*, **3**, 148 (1962).
- ⁵ Katzberg, A. A., and Hendrickx, A. G., *Science, N.Y.*, **152**, 1225 (1966).
- ⁶ De La Lastra, M., Forcelledo, M. L., and Serrano, C., *J. Reprod. Fert.*, **31**, 23 (1972).
- ⁷ Edwards, R. G., and Gates, A. H., *J. Endocr.*, **18**, 292 (1959).
- ⁸ Edwards, R. G., and Fowler, R. E., *J. Endocr.*, **21**, 147 (1960).
- ⁹ Gibson, E. W., Jaffe, S. C., Lasley, J. F., and Day, B. N., *J. Anim. Sci.*, **22**, 858 (1963).
- ¹⁰ Fowler, R. E., and Edwards, R. G., *J. Endocr.*, **15**, 374 (1957).
- ¹¹ Chrétien, F. C., *Ann. Endocrinol.*, **33**, 507 (1972).
- ¹² Gemzell, C. A., Diczfalusy, E., and Tillinger, G., *J. Endocr.*, **18**, 1333 (1958).
- ¹³ Apostolakis, M., Bettendorf, G., and Voigt, K. D., *Acta endocr.*, **41**, 14 (1962).
- ¹⁴ Crooke, A. C., *Br. med. Bull.*, **26**, 17 (1970).
- ¹⁵ Berger, M. J., Taymor, M. L., Karam, K., and Nudenberg, F., *Fertil. Steril.*, **23**, 783 (1972).
- ¹⁶ Robertson, S., and Grant, A., *Aust. N.Z. J. Obstet. Gynaec.*, **12**, 253 (1972).
- ¹⁷ Boué, J. G., and Boué, A., *Lancet*, **i**, 679 (1973).
- ¹⁸ Layton, W. M., and Hallesy, D. W., *Science, N.Y.*, **149**, 306 (1965).
- ¹⁹ Vickers, T. H., *Br. J. expl. Path.*, **53**, 5 (1972).
- ²⁰ Kleinebrecht, J., Degenhardt, K. H., Fränz, J., and Schneider, G., *Teratology*, **5**, 295 (1972).
- ²¹ Grüneberg, H., *The Pathology of Development* (Blackwell, Oxford, 1963).

Identification of Skin in Airborne Particulate Matter

IN the assessment of airborne particulate contamination, it is necessary to identify and quantify the particle species in the environment. Many of the particles recovered from the air have been identified microscopically as being skin scales shed from humans, similar to those shown in Fig. 1. In some circumstances particles may be recovered which are fragmented and appear "dirty" and their microscopic identification then becomes difficult¹. This is especially true when airborne samples from outside air contain small fragments of leaves or the wax cuticle from plant surfaces which are similar in appearance to skin scales.

Here we describe a technique for identifying skin particles by analysis of the surface fats on the recovered particles. In this way many of the airborne particles which have been dismissed as "dust of unknown origin" are shown to have originated from the skin of humans or animals.

The composition of the cuticle and surface waxes on leaves is well documented². In most species, the hydrocarbons in the surface wax are saturated and consist predominantly of substances with an odd number of carbon atoms. In cabbage leaves, for instance, the surface wax consists of a complex mixture of long chain compounds, but some 65% of this wax is made up of only seven different straight chain compounds, all twenty-nine carbon atoms long. One compound, normal-nonacosane (n-C29), makes up more than 90% of the hydrocarbon fraction. Table 1 shows the average composition of human skin surface fat taken from the forearm³.

Our skin identification technique is based on the presence of squalene in human skin and its absence from the surface layer of leaves. (As a preliminary to the analysis of samples of airborne particles, the presence of squalene on the skin surface was confirmed by the use of a Varian Aerograph 2700 gas chromatograph coupled to a Varian MAT 731 mass spectrometer and data were processed by the Varian 100 MS Spectro System.) Skin was gently scraped with a scalpel blade and the dislodged skin scales were collected. The fats were then dissolved in a known quantity of carbon disulphide and an aliquot injected into a Perkin Elmer chromatograph. The squalene content was shown to be of the order of 1% by weight of the collected skin scales (Fig. 2) and the quantities of squalene recovered by petroleum ether washing of the skin of various subjects were

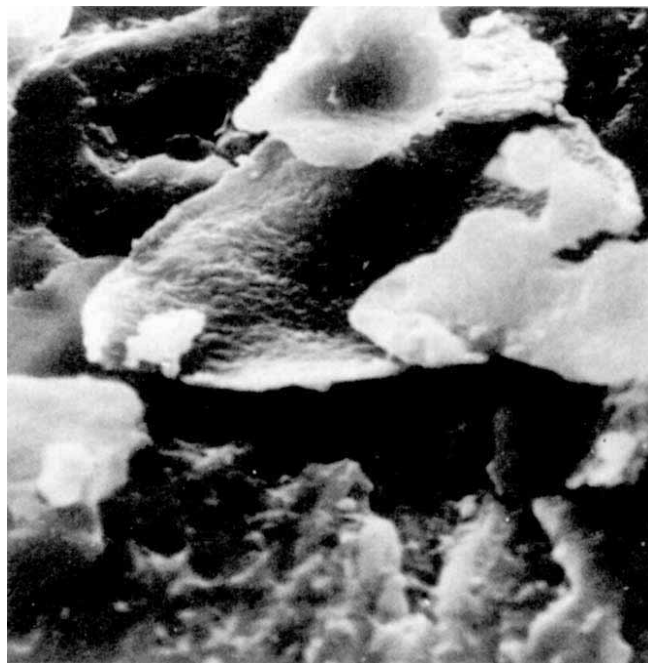


Fig. 1 Scanning electron micrograph of skin fragments some 2 to 5 µm across and 0.5 µm thick, recovered from room air.

Table 1 Composition of Average Skin Surface Fat from the Forearms of Men^a

Free fatty acids	30.0%
Triglycerides	32.5%
Straight chain wax esters	10.7%
Branch chain wax esters	
Cholesterylesters	4.3%
Cholesterol (free)	2.5%
Squalene	5%
Paraffins	7.5%
Unidentified unsaponified fat	7.5%

Table 2 The Percentage of Skin (by Weight) in the Collected Air Samples from Four Locations

Origin of samples	Particle size ranges		
	6 to 2 µm	2 to 0.7 µm	0.7 to 0.4 µm
a Garden	0.4	0.6	0.8
House	0.4	0.7	0.8
Laboratory corridor	1.0	0.5	0.5
b Underground system	5.5 to 3.5 µm 10	3.5 to 2 µm 10	2 to 0.3 µm 10

Airborne particles were recovered from the air of various environments using either, *a*, a Casella Cascade Impactor or, *b*, an Andersen Mini-Sampler. The collection disks in these samplers were thoroughly cleaned with redistilled carbon disulphide before the sample was obtained. The particulate matter was removed from the disks by scraping with a thoroughly clean scalpel and then transferred to a small glass tube. The disks were weighed before and after the particles were removed and 5 µl of re-distilled carbon disulphide was added to the sample of particles from a microsyringe. The solvent and particles were drawn in and out of the syringe needle several times to ensure mixing before being injected into the gas chromatograph.

consistently of the order of 1 µg cm⁻² of skin area washed.

Samples of airborne particulates were collected from the open air of a rural area, indoors in the same rural area, from the corridors of the NIMR Laboratories in Hampstead and from the microenvironment of a passenger travelling in the London Underground system. The collected particles deposited on sampling disks in each of the sampling apparatus so that the fraction on any disk was within a well defined particle size range. Very little material was collected by the samplers for particles

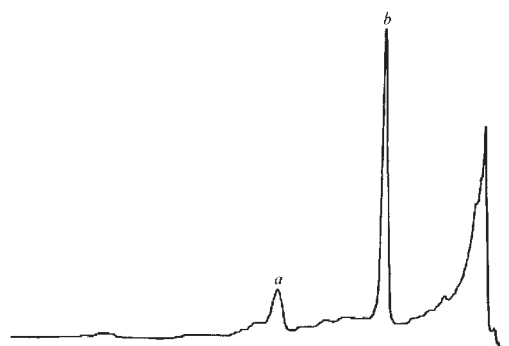


Fig. 2 Chromatogram of the fats on skin scales scraped from the body surface. The gas chromatograph used was a Perkin Elmer F-11 single column instrument with a flame ionization detector and the column was 6 foot × 3 mm internal diameter glass containing OV-1 3% on Gas Chrom Q. All injections were made directly on to the column packing. The oven temperature was 260°C isothermal and the injection port temperature 260°C. The carrier was nitrogen at 10 pounds per square inch inlet pressure. The air pressure was 25 pounds per square inch and the hydrogen pressure was 18 pounds per square inch. The solvent used was re-distilled carbon disulphide. Its purity was checked by a 4 µl injection before each set of samples was run on the chromatograph. Great care was necessary to avoid contamination at all stages of sample handling. (Simply touching the outside of the syringe needle with the fingers would give measurable amounts of squalene on the chromatogram.) All glassware was cleaned in Decon 90, rinsed thoroughly with water, dried and then rinsed with re-distilled carbon disulphide. Random checks were made to ensure the cleanliness of the glassware. *a*, Cholesterol; *b*, squalene.

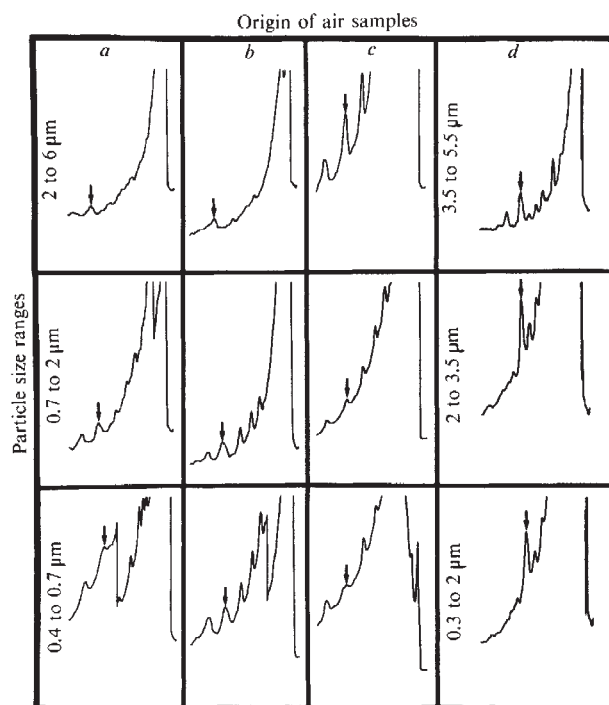


Fig. 3 Chromatograms showing the presence of squalene (arrowed) in air samples from, *a*, garden air; *b*, house air; *c*, laboratory air; *d*, air from the London Underground system.

in the largest size fraction (that is particles greater than 20 µm diameter) and these deposits were not analysed. Figure 3 shows the chromatograms produced from the airborne particulates from the four sampling locations. The percentage of skin (by weight) detected by the chromatograph (given that the squalene weight content of skin is 1%) for these locations is summarized in Table 2.

The percentage of the total collected particle weight, identified as skin by the presence of squalene, in the garden and house air was similar throughout the size range 0.4 to 6 µm, but some five times more air had to be sampled in the garden than in the house to give an adequate sample for analysis.

The total weights of skin found in the two locations are in reasonable accord with other results, to be reported elsewhere, which show that the number concentrations per unit volume of air (in the size range 0.5 to 10 µm) are consistently an order of magnitude greater in the house air than in the garden air when the size spectra of all species of airborne particles are considered.

The gas chromatograph makes possible the detection and quantification of squalene in particles recovered from the air and from the surface fats of the human skin. As this compound is absent from the surface waxes of plant leaves, the technique described here may be used in the assessment of environmental contamination caused by desquamated skin scales.

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¹ Clark, R. P., *J. Physiol.*, **232**, No. 1, 5P (1973).

² Zelitch, I., in *Photosynthesis, Photorespiration and Plant Productivity* (Academic Press, 1971).

³ Wheatley, V. R., *The Chemical Composition of Sebum*, 90 (Livres Jubilaire 1901-51 de la Société Belge de Dermatologie et de Syphiligraphie, 1952).

Carbon Isotope Discrimination in Photosynthesis of CAM Plants

SUCCULENT plants capable of Crassulacean acid metabolism (CAM) show extremely variable carbon isotope discrimination ratios^{1,2}. This ratio, usually expressed as a $\delta^{13}\text{C}$ value referred to a standard³, has emerged as a useful diagnostic criterion to determine photosynthetic pathways in higher plants³⁻⁵. Species with the C_4 photosynthetic pathway⁶ show less negative $\delta^{13}\text{C}$ values than do species with the C_3 photosynthetic pathway. The difference in isotope discrimination is believed to be a result of the different fractionation characteristics of the primary phosphoenolpyruvate (PEP) carboxylase of C_4 plants and primary ribulosediphosphate (RuDP) carboxylase of C_3 plants⁷. Within a species, the $\delta^{13}\text{C}$ value for total carbon does not usually vary by more than $\pm 0.5\text{‰}$, and it is largely insensitive to environmental conditions during growth⁸, although the soluble components within a particular leaf may show some variation⁹. Examples of the constancy of $\delta^{13}\text{C}$ values are shown in the genus *Atriplex*, where C_4 species have less negative values (for total carbon) of -8 to -12‰ than C_3 species which range between -25 and -27‰ (ref. 8).

Here we show that the $\delta^{13}\text{C}$ value for total carbon in CAM plants undergoes significant and predictable responses to controlled growth conditions. The $\delta^{13}\text{C}$ value is correlated with other physiological events to demonstrate that the growth conditions determine the expression of C_3 -like or C_4 -like components of photosynthetic metabolism in CAM plants.

CAM plants such as *Kalanchoe daigremontiana* may fix CO_2 in the light or in the dark. Plants subjected to short hot days and long cool nights rely almost entirely on dark CO_2 fixation for net carbon gain^{10,11}. Carbon dioxide fixation in the dark in CAM plants involves a primary carboxylation of PEP to form oxaloacetate, which is reduced to malate¹². Subsequent metabolism of malic acid to carbohydrate in the light completes the analogy between these temporally separated events and the same spatially separated steps of C_4 photosynthesis^{6,13,14}.

The same CAM species may be grown in conditions of long cool days and short warm nights, in which case CO_2 fixation is largely restricted to the light period^{10,11}. During steady state photosynthesis, *K. daigremontiana* shows a light saturated CO_2 compensation point of about 50 p.p.m. in air (W. G. A., B. O. Austin, and R. O. Slatyer, in preparation). Although malate and aspartate contain a variable proportion of total radioactivity¹⁵ during short term photosynthesis in $^{14}\text{CO}_2$, pulse-chase studies show that the C_4 acids continue to accumulate label in the light (Fig. 1). The C_4 acids are end products of $^{14}\text{CO}_2$ fixation in the light¹³, and are not transitory intermediates as is the case with the C_4 pathway of photosynthesis. The CO_2 compensation point, and the pulse-chase behaviour of 3-phosphoglycerate and sugar phosphates (Fig. 1), suggest that CO_2 fixation in the light in *K. daigremontiana* involves direct carboxylation of RuDP and the C_3 pathway of photosynthesis.

Succulents may change their photosynthetic pathway in response to environmental influences other than temperature. Winter and von Willert¹⁶ discovered that the C_3 plant *Mesembryanthemum crystallinum* may be induced to fix CO_2 in the dark when treated with NaCl. The dark fixation of CO_2 results in a diurnal accumulation of malic acid similar to that found in CAM plants grown under short hot days and long cool nights.

We have used these two techniques, the regulation of day/night length and temperature during growth of *K. daigremontiana* and *K. blossfeldiana*, and the salinization of *M. crystallinum*, to investigate the effects of controlled environment during growth on CO_2 fixation, malate synthesis, and $\delta^{13}\text{C}$ values in CAM plants. Plants were grown in the specified conditions in CSIRO phytotron cabinets or artificially illuminated growth chambers. *M. crystallinum* was grown in soil in a greenhouse for 7 weeks before irrigating some plants with 0.35 N NaCl. Salinized and control plants were assayed 3 weeks later. Carbon dioxide fixation was followed by infrared gas analysis and diurnal changes in acid were estimated by

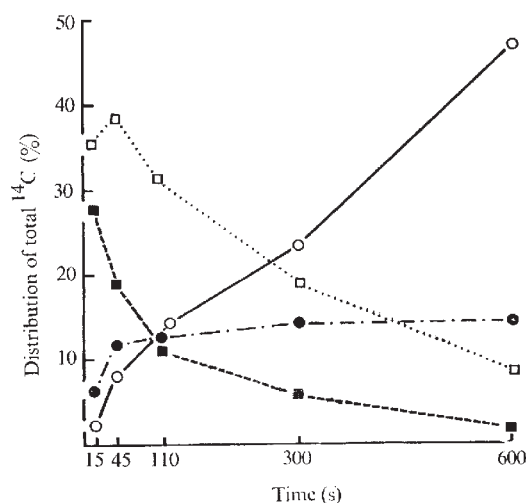


Fig. 1 Percentage distribution of ^{14}C in photosynthetic products extracted from leaves of *K. daigremontiana* exposed to $^{14}\text{CO}_2$ in a 15 s pulse, followed by a chase in $^{12}\text{CO}_2$ for up to 600 s. Leaf material was removed from plants in the $27/15^\circ\text{C}$ regime of experiment 2 (Table 1) at the end of the light period. Leaf segments ($2.5 \times 4\text{ cm}$) were illuminated for 10 min with $30,000\text{ erg cm}^{-2}\text{ s}^{-1}$ of white light at 28°C before exposure to $50\text{ }\mu\text{Ci }^{14}\text{CO}_2$ ($60\text{ }\mu\text{Ci }\mu\text{mol}^{-1}$) in a 75 ml vessel. (●), Malate+aspartate; (○), sugars; (■), sugar monophosphates+3-PGA; (□), sugar diphosphates.

titration and direct assay for malate. The $\delta^{13}\text{C}$ value was determined by mass spectrometry after combustion of dried plant material¹⁷. Five to twenty plants were sampled in each treatment.

Table 1 shows the results of four such experiments. Treatments which permit CO_2 fixation predominantly in the light period (C_3 -like photosynthetic mode) result in small changes in malic acid content and yield more negative $\delta^{13}\text{C}$ values. Treatments which restrict CO_2 fixation predominantly to the dark period (C_4 -like photosynthetic mode) result in large changes in malic acid content and less negative $\delta^{13}\text{C}$ values. It is unlikely that the increased malic acid content is in itself responsible for the more negative $\delta^{13}\text{C}$ values, which presumably apply to all components of the tissue⁹. Duplicate determinations of the $\delta^{13}\text{C}$ value in experiments 2 and 3 show the reliability of the method when based on a large number of plant samples.

There is a good correlation between the principal mode of CO_2 fixation during growth and the $\delta^{13}\text{C}$ values. Growth dependent on dark fixation results in less negative $\delta^{13}\text{C}$ values than growth which is largely dependent on CO_2 fixation in the light. The magnitude of the change in $\delta^{13}\text{C}$ value, and absolute $\delta^{13}\text{C}$ values, display some variation between experiments and species which may be explained in terms of the conditions of the experiments. In experiment 1, for example, growth of *K. daigremontiana* on the $20/25^\circ\text{C}$ regime was five-fold greater than that on the $30/15^\circ\text{C}$ regime. In experiment 4, *M. crystallinum* was grown for 7 weeks in control conditions before treatment with 0.35 N NaCl. The proportion of tissue carbon laid down in the C_4 -like growth conditions of both of these experiments is smaller than that laid down in the C_3 -like conditions. The changes in $\delta^{13}\text{C}$ values in the C_4 -like treatments are thus considerably underestimated.

We conclude that CAM plants have the option of a C_3 -like or a C_4 -like mode of photosynthetic metabolism. In most conditions it is likely that a CAM plant will receive contributions from both components during its development. The proportion gained in the light or dark responds rapidly to changes in temperature on a day to day basis, as shown by Neales¹¹. The final $\delta^{13}\text{C}$ value shown by the tissue should reflect the proportion of carbon gained through each pathway during growth.

Table 1 Effects of Controlled Growth Conditions on the Photosynthetic Behaviour of Succulent Plants Capable of Crassulacean Acid Metabolism

Species and tissue	Growth period (weeks)	Temperature day/night (°C)	Day length (h)	Illumination	Photosynthetic mode	Diurnal malate change ($\mu\text{mol g}^{-1}$ fresh wt)	$\delta^{13}\text{C}$ value (‰)
1. <i>K. daigremontiana</i> plantlets	0	—	—	—	—	—	—20.1
young plants	3	20/25	8	sunlight	C ₃ -like	25	—24.0
young plants	3	30/15	8	sunlight	C ₄ -like	53	—19.8
2. <i>K. daigremontiana</i> mature leaves	10	24/19	16	sunlight + artificial*	C ₃ -like	20	—18.0, —18.4
mature leaves	10	27/15	12	artificial†	C ₄ -like	110	—13.3, —13.3
3. <i>K. blossfeldiana</i> mature leaves	12	26/20	15	sunlight + artificial*	C ₃ -like	‡	—26.4, —26.4
mature leaves	12	27/17	9	artificial†	C ₄ -like	‡	—19.1, —18.4
4. <i>M. crystallinum</i> control, mature leaves	10	glasshouse conditions		sunlight	C ₃ -like	0.1	—28.3
0.35 M NaCl, mature leaves	10	glasshouse conditions		sunlight	C ₄ -like	38	—22.5

* Approximately $6 \times 10^5 \text{ erg cm}^{-2} \text{ s}^{-1}$ (400–700 nm) with day length extended with low intensity tungsten lamps.

† Approximately $3 \times 10^4 \text{ erg cm}^{-2} \text{ s}^{-1}$ (400–700 nm) from tungsten lamps and fluorescent tubes.

‡ Not measured here, see ref. 10.

Our experiments show that a variety of environmental conditions may regulate the proportion of carbon gained by either pathway. It is likely that the availability of water may be of paramount importance. Kluge and Fischer¹⁸ showed that droughting *Bryophyllum tubiflorum* rapidly reduced its ability to fix CO₂ in the light (the C₃-like component). Consistent with these observations, we have found that droughting mature *K. daigremontiana*, grown on the 20/25° C treatment of experiment one, made the $\delta^{13}\text{C}$ value less negative. After 9 weeks of drought, the control $\delta^{13}\text{C}$ value of $-22.8 \pm 0.8\text{‰}$ moved to $-16.2 \pm 0.3\text{‰}$ as the plants relied increasingly on the dark CO₂ fixation; the C₄-like component of photosynthesis. Similar data have been obtained for the $\delta^{13}\text{C}$ values of *Optunia inermis* throughout its range in eastern Australia; in arid regions of central southern Queensland, $\delta^{13}\text{C}$ values for this species range between -11 and -13‰ and in the more temperate, higher rainfall areas of coastal southern New South Wales we have found values of -16 to -18‰ . Environmental control of the photosynthetic options of these plants has brought about predictable changes in the $\delta^{13}\text{C}$ value.

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¹ Bender, M. M., *Phytochemistry*, **10**, 1239 (1971).

² Lerman, J. C., in *Proc. Eighth Int. Conf. Radiocarbon Dating* (in the press).

³ Smith, B. N., and Epstein, S., *Pl. Physiol. Lancaster*, **47**, 380 (1971).

⁴ Tregunna, E. B., Smith, B. N., Berry, J. A., and Downton, W. J. S., *Can. J. Bot.*, **48**, 1209 (1970).

⁵ Troughton, J. H., in *Proc. Eighth Int. Conf. Radiocarbon Dating* (in the press).

⁶ *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.) (Wiley-Interscience, New York, 1971).

⁷ Smith, B. N., *Bioscience*, **22**, 226 (1972).

⁸ Berry, J., Troughton, J. H., and Björkman, O., *Carnegie Inst. Wash. Yb.*, **71**, 158 (1972).

⁹ Whelan, T., Sackett, W. M., and Benedict, C. R., *Biochem. biophys. Res. Commun.*, **41**, 1205 (1970).

¹⁰ Queiroz, O., *Physiol. Vég.*, **3**, 203 (1965).

¹¹ Neales, T. F., *Aust. J. Biol. Sci.* (in the press).

¹² Sutton, B. G., and Osmond, C. B., *Pl. Physiol. Lancaster*, **50**, 360 (1972).

¹³ Kluge, M., in *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.), 283 (Wiley-Interscience, New York, 1971).

¹⁴ Ting, I. P., in *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.), 169 (Wiley-Interscience, New York, 1971).

¹⁵ Avadhani, P. N., Osmond, C. B., and Tan, K. K., in *Photosynthesis and Photorespiration* (edit. by Hatch, M. D., Osmond, C. B., and Slatyer, R. O.), 288 (Wiley-Interscience, New York, 1971).

¹⁶ Winter, K., and von Willert, D. J., *Z. Pflanzenphysiol.*, **67**, 166 (1972).

¹⁷ Craig, H., *Geochim. Cosmochim. Acta*, **3**, 53 (1953).

¹⁸ Kluge, M., and Fischer, K., *Planta (Berl.)*, **77**, 212 (1967).

Accelerated Germination by Osmotic Seed Treatment

CROP seeds sown in cold soil are notoriously slow to emerge and it would obviously be desirable to shorten the time from sowing to seedling emergence and the time between the emergence of the first and the last seedling. This has occasionally been achieved by the pre-sowing seed treatment sometimes referred to as 'hardening'¹⁻³ or 'advancing'⁴ involving repeated cycles of imbibition of a carefully controlled quantity of water, followed by drying back the seeds before the radicles emerge but results have not been consistent. There have been some successful attempts to replace this method by imbibition of seeds in salt solutions⁵⁻⁷; tomato seeds thus treated for 6 d exhibit a much higher level of RNA production during germination than untreated seeds⁸.

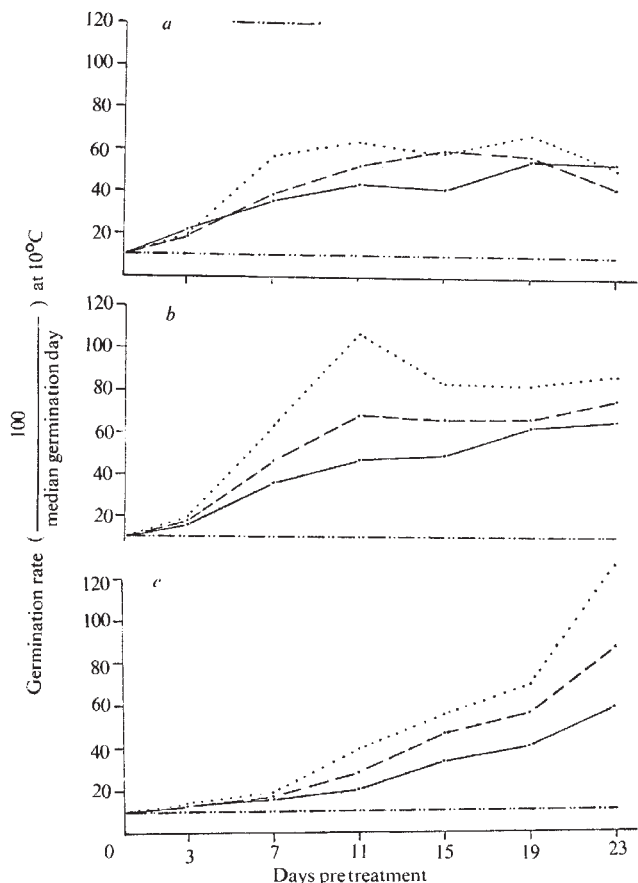


Fig. 1 Germination rate of onion seeds in response to osmotic pretreatment. Effect of water potential, temperature and duration of treatment. Seeds were placed on filter papers moistened with solutions of a polyethylene glycol of high molecular weight, Carbowax 6000 (Union Carbide) of -10 , -12.5 or -15 bar osmotic potential⁹ and at temperatures of 10 , 15 and 20°C in the dark (batches of fifty seeds on four Whatman No. 1 filter papers in 8 ml of solution per 9 cm diameter Petri dish). After 3, 7, 11, 15, 19 and 23 d, four replicates of each of the treatments were thoroughly washed and surface dried. The seeds were then placed in other Petri dishes similarly prepared but with distilled water and were allowed to germinate at 10°C in the dark. . . . , -10.0 bar osmotic potential; - - - , -12.5 bar osmotic potential; — — — , -15.0 bar osmotic potential; — · — · — , untreated. *a*, 20°C ; *b*, 15°C ; *c*, 10°C .

By using a polyethylene glycol of high molecular weight, we have avoided the complications of both 'hardening' and the use of salts. Promising results have been obtained in preliminary experiments on carrot (*Daucus carota* L.) and red beet (*Beta vulgaris* L.), but we report here only on onion (*Allium cepa* L.) seeds.

All osmotic pretreatments resulted in an increase in the rate of radicle emergence (Fig. 1) depending in magnitude on

all three components of the preparatory treatment, that is, osmotic potential, temperature and duration. There was no effect on the percentage of seeds germinated but certain treatment combinations permitted germination of some seeds in the osmotic solution (Table 1).

For every temperature and osmotic potential combination, increasing treatment duration initially increased the rate of subsequent germination. At 15°C and 20°C , however, an optimal duration was found beyond which there was no further benefit and in some cases an eventual decline. Almost all comparable rates of germination were lower after treatment at 20°C than 15°C . At 10°C the optimal duration may not have been reached in this experiment.

When seeds were treated at 10°C and 15°C , a lower osmotic potential resulted in a lower rate of germination and a longer treatment was needed for the seeds to reach any desired rate of germination. Treatment at the lowest temperature caused a slower increase in the rate of germination than treatment at the higher temperatures, but ultimately resulted in the highest rate achieved. The combination of -10 bar and 15°C for 11 d, however, was nearly as successful in increasing the rate of germination of the seed population as the best treatment; -10 bar at 10°C was 0.8 d, when the resulting median time from sowing to radicle emergence at a germination temperature of 10°C was 0.8 d, which compared with 9.3 d for untreated seeds (Fig. 2). It would be hard to better this result.

This technique has fascinating physiological implications,

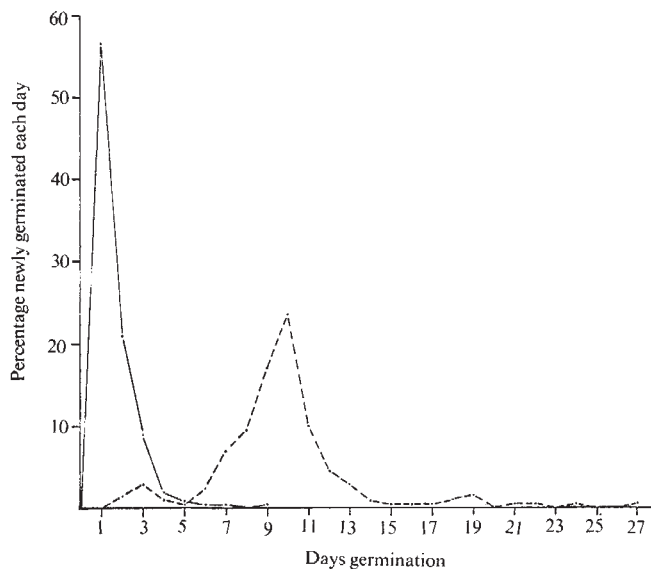


Fig. 2 Germination of onion seeds in response to 23 d osmotic pretreatment in PEG (Carbowax 6000) solution of -10 bar potential at 10°C . Germination at 10°C on filter paper moistened with water. The pretreatment increases both the rate and the uniformity of germination. —, Pretreated, washed and dried; - - -, untreated.

Table 1 Percentage Germination of Onion Seeds (cv. Excellent) During and After Osmotic Pretreatment* in PEG (Carbowax 6000)

Pretreatment		Days					
Temp. ($^{\circ}\text{C}$)	Potential (bars)	3	7	11	15	19	23
10	-10	0+96.0	4.0+88.0	9.0+83.5	10.5+82.0	15.0+79.0	9.0+80.5
	-12.5	0+93.5	1.0+91.0	0+91.5	6.0+86.0	2.0+85.5	3.0+88.5
	-15	0+90.5	0+94.5	0.5+93.0	1.5+92.0	1.0+85.5	1.5+89.5
15	-10	0+87.5	1.5+93.5	4.5+90.5	15.0+82.0	21.5+72.5	18.0+74.5
	-12.5	0+92.0	0+92.0	1.5+90.0	5.0+88.5	2.5+94.0	4.5+93.0
	-15	0+93.0	0+90.0	0+94.0	2.5+92.5	1.0+94.5	1.0+90.0
10	-10	0+93.5	0+92.5	0.5+94.0	0+88.5	5.5+84.5	3.5+91.0
	-12.5	0+92.0	0+90.0	0+89.5	0+89.5	0+90.5	0.5+94.0
	-15	0+96.0	0+93.0	0+92.0	0.5+90.5	0+89.5	0+88.0

Untreated 90.0.

* Different water potentials, temperatures and durations of treatment. Germination temperature 10°C .

and promises to be of considerable value in establishing crops from seeds sown in early spring.

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- ¹ Henckel, P. A., *A. Rev. Plant Physiol.*, **15**, 363 (1964).
- ² Austin, R. B., Longden, P. C., and Hutchinson, Jane, *Ann. Bot.*, **33**, 883 (1969).
- ³ Hegarty, T. W., *Hort. Res.*, **10**, 59 (1970).
- ⁴ Longden, P. C., *J. agric. Sci.*, **77**, 43 (1971).
- ⁵ Levitt, J., and Hamm, P. C., *Plant Physiol.*, **18**, 288 (1943).
- ⁶ Ells, J., *Proc. Am. Soc. hort. Sci.*, **83**, 684 (1963).
- ⁷ Oyer, E. B., and Koehler, D. E., *Proc. 17th Int. Hort. Cong., Maryland*, **1**, 626 (1966).
- ⁸ Koehler, D. E., thesis, Purdue University, USA (1967).
- ⁹ Manohar, M. S., *Planta (Berl.)*, **71**, 81 (1966).

In vitro Control of *de novo* Flower, Bud, Root, and Callus Differentiation from Excised Epidermal Tissues

AMONG the plant species capable of neoformation, a much larger number are capable of root and vegetative bud formation than of direct flower neoformation. For most materials, the formation of buds or roots or flowers concerns different tissues, such as epidermis, perivascular or callus tissue of cambial origin, and each tissue maintains complex correlation with the surrounding tissues.

The study of the control of organogenetic differentiation is faced with certain difficulties because of the different tissues involved in the origin of different types of organogenesis and the complexity of the material: lack of criteria for a valid study of changes during organogenesis and for rigorous comparisons among the different types of organogenesis.

This problem was simplified when it was found that a few superficial cell layers excised and cultured *in vitro* have organogenetic potentialities which can be controlled at will. From very small explants of *Nautilocalyx lynchei* (5 mm × 2 mm), composed of one to five layers of foliar epidermal and subepidermal cells, mitoses without organogenesis (non-organogenetic differentiation), mitoses leading to the controlled formation of roots (Fig. 1) or vegetative buds (Fig. 2) (organogenetic differentiation) were induced from the same cellular layer, the epidermis, each cell of which divides¹. Thus, by the *in vitro* culture of a few superficial cell layers, it is possible to show experimentally the capacity for callus formation and *de novo* formation of roots up to then apparently inhibited. In leaf or stem fragments, root formation is confined to perivascular tissue.

With such materials, certain fundamental problems in organogenesis, which are difficult to study using relatively large organ fragments, can be examined. For example, for *Nautilocalyx*, where bud and root neoformations arise from epidermal cells, it would be possible to study the evolution and distribution of organogenetic centres by observing the thousands of cells on the epidermal surface to determine whether there is a special mode of distribution of these centres in relation to the presence of basal hair cells or stomata.

To the potentialities for bud, root and callus formation, we were able to add the direct *de novo* formation of floral buds on the explant without intermediate callus or leaf

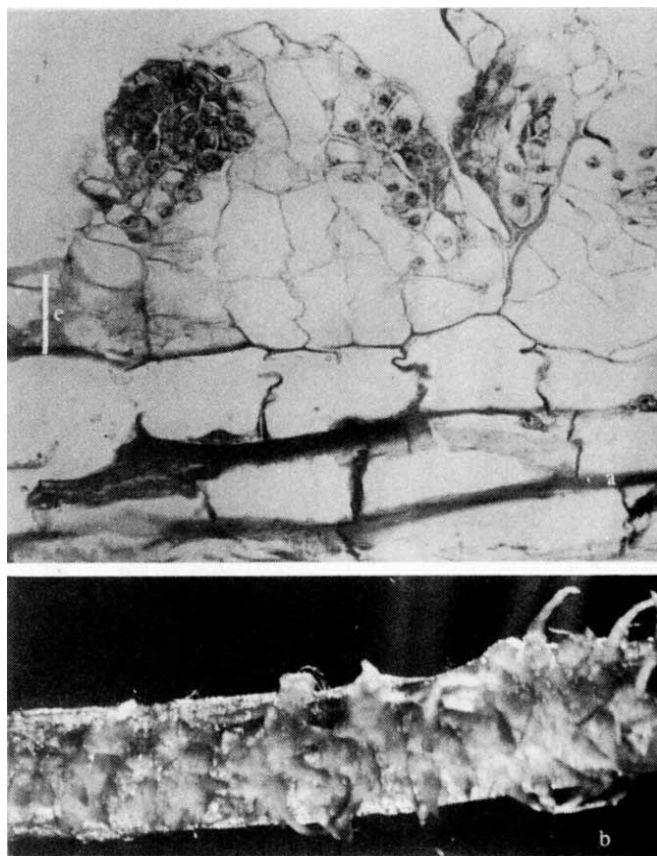


Fig. 1 a, Longitudinal section of an explant of *Nautilocalyx* showing the epidermal origin of the root primordia organized *de novo* from divided epidermal cells. (×160.) b, *De novo* formation of roots from epidermal tissue explants after 37 d. e, Epidermis.

formation by applying the techniques described for *Nautilocalyx* to explants composed of three to five layers of epidermal and subepidermal cells excised from floral ramifications of *Nicotiana tabacum* "W.38". These explants are excised with a scalpel from floral ramifications previously disinfected in a 7% hypochlorite solution for 10 min, and planted on an aseptic culture medium composed of mineral elements, vitamins, auxin, cytokinin and sucrose.

Depending on the relative concentrations of the auxin, cytokinin and sucrose and on certain environmental factors, such as light and temperature, different types of differentiation can be induced in 10 to 14 d: callus formation which has been maintained without organogenesis for 2 yr, root formation, vegetative shoot formation and floral bud formation (Fig. 3). This last type of organogenetic differentiation is the most interesting. It implies that stimulation of mitoses takes place in cells which had lost

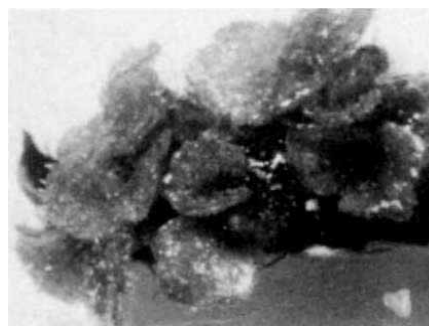


Fig. 2 Explant showing the *de novo* shoot formation, the surface covered with buds after 37 d. (×8.)

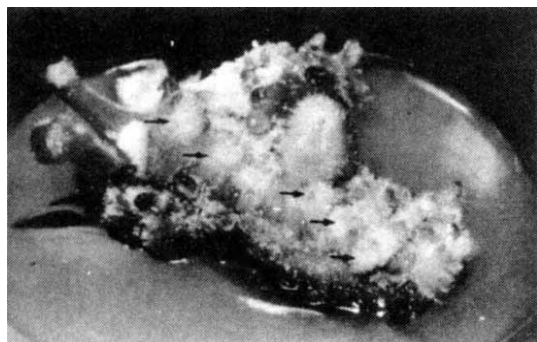


Fig. 3 Surface of the explant covered with floral buds formed *de novo* (arrows). 20 d stage. ($\times 2.5$)

their capacity to divide, and that, after a short phase of production of floral parts, meiosis occurs.

Up to a certain stage, each one of these types of organogenesis can be converted into a different one. After this point, differentiation is irreversible.

An analysis at the cellular level has established that all these types of differentiation originate from the same cellular layer, the subepidermal layer, each cell of which participates in the differentiation. It is thus relatively easy to study the cellular and molecular modifications which occur in this layer during each type of organogenetic differentiation or at the exact moment of conversion from one programme to another.

These new experimental systems, with others developed in our research group, will facilitate a more precise approach to the determinism of differentiation in pluricellular systems by their simplicity and homogeneity.

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¹ Tran Thanh Van, M., and Drira, A., *Colloques int. Cent. natn. Rech. scient.*, **193**, 169 (1970).

Locomotor Affinities of *Hominoid* Tali from Kenya

A CONTRIBUTION to this journal¹ is among several that have attracted criticism from Oxnard². This communication has two objects; first, to reply to comments by Oxnard which relate to a possible ambiguity in the earlier communication; second, to present the results of a new analysis which provide additional evidence for the interpretation of the mode of locomotion of East African dryopithecines.

The object of the earlier work was to test the hypothesis, formulated by the late L. S. B. Leakey³, that two tali, one found at Songhor (CMH 145) and the other at Rusinga (CMH 147), may belong to the taxon "*Kenyapithecus africanus*", a putative early hominid. A multivariate canonical analysis, using extant hominid and pongid comparative material⁴, was performed. The first canonical axis produced clustering of the groups which

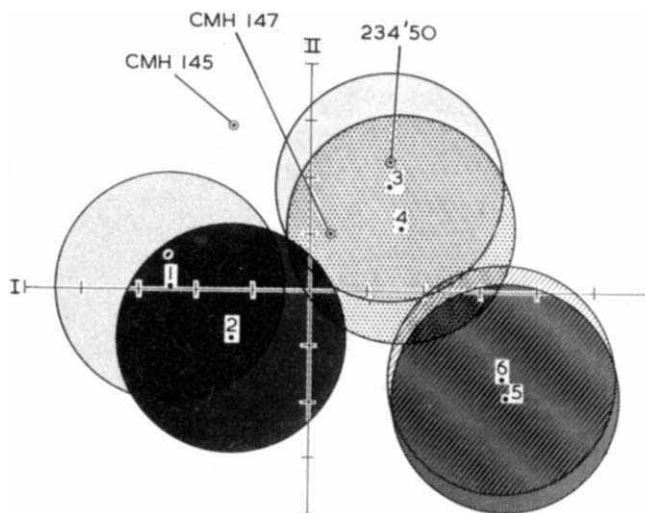


Fig. 1 Plot of canonical axes I and II. Circles are drawn with a radius of two eigenvalues. 1, *Gorilla*; 2, *Pan*; 3, *Papio*; 4, *Cercopithecus* (*mona* and *nictitans*); 5, *Colobus*; 6, *Pongo*.

corresponded to accepted locomotor classifications based on anatomical and behavioural analyses. This demonstrated the efficacy of the canonical coefficients of the first axis as locomotor discriminators. By contrast, the second and higher canonical axes failed to distinguish between "knuckle-walking" apes and bipeds. Thus, *a posteriori*, the canonical coefficients of the second and higher axes had no individual usefulness as discriminants between the extant locomotor groups.

The canonical coefficients for the first canonical axis were then used to process the data for the two fossil tali and an additional talus from Rusinga (234'50), and it was found that these tali lay closer to the knuckle-walking locomotor category than to the bipeds. This was taken as evidence that complemented and substantiated earlier morphological assessments that these tali are pongid or dryopithecine and not hominid.

Oxnard has suggested that the D^2 values indicate the affinity of the fossil tali for the knuckle-walking locomotor group confined to the first canonical axis, and is dependent on the limited choice of comparative groups. He continues, "the *Proconsul* tali are completely unlike African apes rather than similar as previously assessed", presumably because the sum total of the individually insignificant deviations in the second and higher axes place the fossils several units of Generalized Distance away from the extant apes.

Therefore, in order to investigate further the functional relationships of these fossil tali, and to attempt to reconcile these seemingly opposed views, a new canonical analysis has been performed.

The new comparative groups, with their locomotor classifications, are listed in Table 1. An attempt has been made, within the limits of available material, to improve the resolution of this technique by including a wider variety of sub-human Old World primate locomotor modes. Mahalanobis D^2 distances and canonical variates were calculated from measurements on the tali as outlined previously⁴. Seven significant canonical axes, of which the first three contained 97% of the total variability, were produced; subsequent axes did not individually contribute significantly to the discrimination.

Table 1 Comparative Groups

Groups	1 <i>Gorilla</i>	2 <i>Pan</i>	3 <i>Papio</i>	4 <i>Cercopithecus</i> <i>mona/nictitans</i>	5 <i>Colobus</i>	6 <i>Pongo</i>
No. of specimens	50	50	42	17	16	15
Locomotor classification	Knuckle walker	Knuckle walker	Quadruped	Quadruped	Quadruped (with leaping)	Brachiator (hind limb grasping)

Table 2 D^2 Distances and Probability Levels between Group Means

Groups	1	2	3	4	5
2	3.4 ($<0.1\%$)				
3	18.8 ($<0.1\%$)	14.9 ($<0.1\%$)			
4	18.2 ($<0.1\%$)	14.2 ($<0.1\%$)	1.6 (1.0%)		
5	47.4 ($<0.1\%$)	32.7 ($<0.1\%$)	25.4 ($<0.1\%$)	20.5 ($<0.1\%$)	
6	47.1 ($<0.1\%$)	34.5 ($<0.1\%$)	26.9 ($<0.1\%$)	21.2 ($<0.1\%$)	34.3 ($<0.1\%$)

Table 3 D^2 Distances and Probability Levels between Groups and Special Cases

	1	2	Comparative groups		5	6
Fossil tali	<i>Gorilla</i>	<i>Pan</i>	3 <i>Papio</i>	4 <i>Cercopithecus</i>	<i>Colobus</i>	<i>Pongo</i>
CMH 145	13.7 (5.0%)	18.4 (1.0%)	13.5 (5.0%)	14.8 (5.0%)	64.2 ($<0.5\%$)	50.5 ($<0.5\%$)
CMH 147	12.2 (10.0%)	8.7 (25.0%)	3.5 (85.0%)	5.7 (50.0%)	26.5 ($<0.5\%$)	30.6 ($<0.5\%$)
234'50	21.3 ($<0.5\%$)	17.7 (1.0%)	0.5 (99.0%)	2.5 (90.0%)	29.6 ($<0.5\%$)	29.7 ($<0.5\%$)

A plot of canonical axes I and II (Fig. 1) shows a clear separation of the knuckle-walking and quadrupedal locomotor groups, with no such separation between *Pongo* and *Colobus*. A further plot of axes II and III (Fig. 2) shows that axis III is a strong discriminator between *Pongo* and *Colobus* but has little effect on the other groups. Thus, between them these three axes produce a three-dimensional separation of extant samples that is consistent with current locomotor classifications; tali coming from primates with different locomotor modes are rarely confused in this analysis.

Subject to certain limitations, as outlined by Gower⁵, the canonical coefficients derived from the analysis can be used to determine the position of samples of extinct fossil primate genera in the same multivariate space.

The positions of the fossil tali have been plotted for the first three axes. In axis I (which effectively discriminates between a knuckle-walking and quadrupedal gait) two of the fossil tali are clustered with the quadrupeds. The third talus, CMH 145, is not associated with any extant locomotor group. In axis III, which successfully separates *Pongo* and *Colobus* from each other, from the quadrupeds and from the knuckle walkers, all three fossils

eschew the former two groups and cluster with the combined knuckle-walking and quadrupedal groups. In axis I particularly, CMH 145 behaves differently from the other two fossil tali.

The Mahalanobis D^2 distances between the group means, with their probability levels, are given in Table 2; the D^2 distances of the fossil tali from the extant group means, along with probability levels, are given in Table 3. These distances confirm the dissimilarity between the fossil tali and those of both *Pongo* and *Colobus*. One talus, CMH 145, has no close affinity with any of the extant locomotor groups. The other two tali are closely related to tali of the quadrupedal group (*Papio* and *Cercopithecus*) and one, CMH 147, shows some affinity with the knuckle-walking tali.

Thus, by focusing attention on how the talus varies between locomotor modes in a more extensive series of sub-human primate genera than were used in the earlier analysis, two of the fossil tali appear to show the same morphological features as cercopithecoid quadrupeds. These quadrupeds are characterized by a relatively long anterior tarsal segment, associated with the use of the metatarsal heads as the fulcrum for their propulsive movement. Their body weight is transmitted almost exclusively by the trochlear surface of the talus, whereas in the ground-adapted pongids the laterally projecting fibular articular facets, with corresponding modifications of the fibula, indicate a lateral shift in the axis of weight transmission. When the canonical weights are examined for variate I, a long talar neck and an insignificant fibular facet are indeed parameters which discriminate between the quadrupeds and the knuckle-walkers.

In summary, this analysis confirms that these fossil tali are more like the tali of knuckle walkers than bipeds. As suggested by Oxnard, however, the similarity has been shown to be relative because finer locomotor discrimination reveals that the two fossil tali from Rusinga show a greater, and indeed significant, affinity with tali of the quadrupeds *Papio* and *Cercopithecus*. This confirms the earlier morphological assessment of Le Gros Clark and Leakey⁶ and suggests that the inclusion of these Miocene tali in the locomotor group represented by extant knuckle-walking pongids may be premature. Moreover, the behaviour of CMH 145 in the analysis is additional evidence for Pilbeam's contention⁷ that this specimen may belong to a different species from that of the tali from Rusinga.

I thank Professors E. H. Ashton and M. H. Day for their helpful comments, Mr G. B. Newman for continued statistical advice and Miss Leader for typing the manuscript.

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¹ Day, M. H., and Wood, B. A., *Nature*, **222**, 591 (1969).

² Oxnard, C. E., *Am. J. Phys. Anthropol.*, **37**, 3 (1972).

³ Leakey, L. S. B., *Nature*, **213**, 155 (1967).

⁴ Day, M. H., and Wood, B. A., *Man*, **3**, 440 (1968).

⁵ Gower, J. C., *Biometrika*, **55**, 582 (1968).

⁶ Le Gros Clark, W. E., and Leakey, L. S. B., *The Miocene Hominoidea of East Africa, Fossil Mammals of Africa*, No. 1 (British Museum (Natural History), London, 1951).

⁷ Pilbeam, D., *Nature*, **223**, 648 (1969).

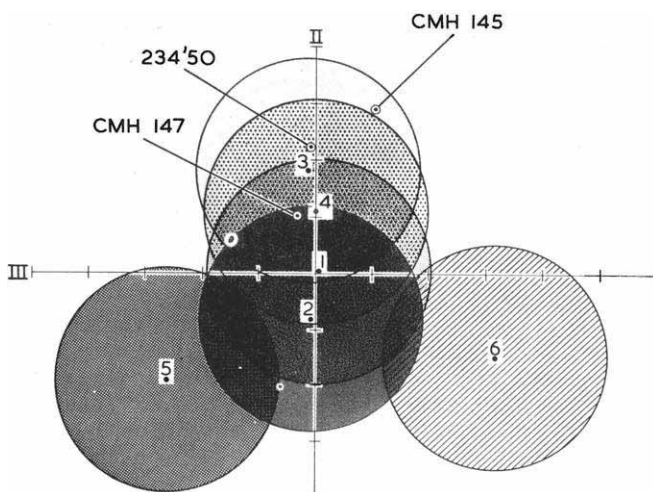


Fig. 2 Plot of canonical axes II and III. Circles are drawn with a radius of two eigenvalues. Comparative groups as in Fig. 1.

BOOK REVIEWS

Primate Evolution

Evolution of Primates: An Introduction to the Biology of Man. By A. B. Chiarelli. Pp. xi+354. (Academic: New York and London, April 1973.) £7.20.

THE publishers announce this as "probably the only comprehensive treatment on primate evolution to be compiled", which is odd because Academic Press have recently issued a much more useful book on virtually the same subject^{1,2}; and most of the wide field approached by Professor Chiarelli is adequately covered in a well written textbook which has already seen several years of service³. It is, indeed, an enormous undertaking to deal with genetics (from the molecular level upwards), speciation, living and fossil primates and the emergence of man all in one volume. However, there is always the danger with such a wide-ranging approach that depth of treatment and command of theoretical issues will be wanting, and Professor Chiarelli has unfortunately failed to cope with the task that he set himself.

Although concerned with the central theme of primate evolution, there is no discussion in the text of the methods used to establish evolutionary relationships. The fundamental concepts of homology and convergence, for example, are left unmentioned. Instead, Chiarelli uses the simple model of the living primates as a graded series of arrested ancestral stages: "In fact, prosimians, monkeys and anthropoid apes may represent the survival of stages reached in the past by human ancestors". In line with this, it is also stated that "comparative anatomists and palaeontologists now generally agree that the tree shrew can serve, *cum grano salis*, as an example of the first phase of the evolution of the primates". First, it is a vast oversimplification to imply that tree shrews simply ceased to evolve some 70 million years ago. Second, it should at least be mentioned that several authorities⁴⁻⁷ have now rejected the hypothesis that tree shrews are related to primates.

Even if used as a model for primate evolution, tree shrews are of marginal importance; but it is nevertheless desirable to describe them accurately. Yet the fossil *Anagale*, which is in any case an unlikely relative of tree shrews^{8,9}, is vaguely referred to as "Oligocene fossil *Ptilocercus* from Mongolia". And when it is stated that the living tree shrews are "among the smallest existing mammals, very lively and aggressive,

and as their metabolic rate is very high they eat voraciously", it is apparent that Chiarelli has confused the Soricidae with the Tupaiidae. We are also told that the tree shrews have eyes "placed at right angles to each other".

Slips of this kind are, unfortunately, abundant. The aye-aye, for instance, is said to live on the juice sucked from sugar-cane, and this admittedly bizarre lemur is granted two different dental formulae on pages 59 and 157. We are informed that, for the lemurs in general, "their intellectual capacity has not increased during their evolution from the time they arrived in Madagascar", and it is stated that Palaeocene and Eocene fossil relatives have been found in Africa. Palaeontologists will also be surprised to see that the map of world distribution of fossil primates omits the entire New World.

Although the description of the better known simians is generally more accurate, it is unsettling to find that Chiarelli—apparently on the basis of superficial karyological and serological similarities—takes the unusual step of classifying the gibbon with cercopithecoid monkeys rather than with the apes. Interpretation of similarities of this kind is by no means simple, and in an intended textbook such sweeping revision of a widely used classification deserves full discussion. In any event, on page 141 Chiarelli overlooks his revision and reverts to placing the gibbon (misprinted as *Hyloteles*) in the Hominoidea.

The sphere of hominid evolution fares no better. On page 236, a maxilla attributed to *Ramapithecus* is mislabelled and printed upside down, and Chiarelli states (perhaps through the agency of yet another misprint) that this genus displays the most hominoid characteristics within the dryopithecine radiation. With the later hominids, in spite of the fact that many recent authors have followed a greatly clarified scheme^{10,11}, Chiarelli revives the now obsolete proliferation of genus and species names. Apart from referring to *Homo erectus* exclusively as "*Pithecanthropus*" and to the *H. erectus*/*H. sapiens* transition stage as "*Protanthropus*", the term "*Palaeanthropus*" is used as if it were a genus name for forms which are also referred to as *Homo* species. Nowadays, this kind of confusion over nomenclature is only justifiable for forms of questionable taxonomic status, such as "*Homo habilis*"

from Olduvai; yet this particular form is totally omitted from the discussion of hominid evolution.

Chiarelli's primary research area is that of genetics and karyology, and chapters on these aspects to some extent compensate for failings elsewhere. Even these chapters, however, are not without serious faults. For example, the discussion of chromosomal evolution which is given with remarkable brevity in chapter 2 is not followed until chapter 11 with the actual evidence for primates. The resulting gap in the train of thought is compounded by errors of omission. There is no definition of metacentric or submetacentric chromosomes, though distinction of these from acrocentrics is fundamental to the understanding of chromosomal evolution. Again, Chiarelli resorts to the concept of arrested ancestral stages in referring to evolutionary processes: "In fact, although it is not possible to study the karyotype of the ancestors of living species, the study of the karyotype of the latter furnished many examples of the stages through which karyotypes have passed during their evolution".

Similar deficiencies occur in the treatment of genetics. For example, two maps (on pages 113 and 115) indicate totally different IB gene frequency distributions in Europe for the ABO blood group system. It is also stated that "the number of amino acids which make up a protein normally does not exceed 20-25", which will misleadingly indicate to some readers that all protein chains are very short, rather than that there are usually 20-25 different kinds of amino acid involved. Again, a fairly competent description of haemoglobin evolution is spoiled on page 105 by confusion over mutation rates and misprinting of the Greek symbols for haemoglobin chains. Finally, for the really crucial developing issue of tree-building using basic biochemical data, the reader is supposed to decipher the following: "In fact, at first approximation, the amount of divergence between two species can be measured by counting the ratio of the number of homologues that have a different amino acid".

There is always a possibility that recitation of individual errors may mask the true merits of a book, and it must in all fairness be said that Professor Chiarelli has exhibited an enormous range of interests and a very broad (if inaccurate) spectrum of knowledge. Two facts are certain, however; treatment of some of the subjects indicates a quite inadequate grasp of the material, and the book as a whole has been pro-

duced with a surprising lack of attention to detail. There are numerous errors of fact, partly because of the large number of printing faults, and the translation is in places not entirely clear. Even the arrangement of the chapters seems to be somewhat haphazard ("The Concept of the Species" and "Evolution of Species" are unaccountably separated by "The Living Primates"). Finally, there is frequent omission of key references from the lists at the ends of the chapters, numerous figures are reproduced without acknowledgment of the source, and the index is insufficient. In short, in spite of the valiant attempt to cover an enormous field, this book is so poorly produced that it will merely serve to confuse students taking courses in primatology.

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- ¹ *Nature*, **243**, 175 (1973).
- ² Hill, W. C. O., *Evolutionary Biology of the Primates* (Academic Press, London and New York, 1972).
- ³ Buettner-Janusch, J., *Origins of Man* (Wiley, New York, 1966).
- ⁴ Romer, A. S., *Vertebrate Palaeontology*, 3rd ed. (University of Chicago Press, Chicago, 1966).
- ⁵ McKenna, M. C., *Folia primat.*, **4**, 1 (1966).
- ⁶ van Valen, L., *Evolution*, **19**, 137 (1965).
- ⁷ Simons, E. L., *Primate Evolution* (Collier-Macmillan, New York, 1972). See also Martin, R. D., *Man*, **3**, 377 (1968).
- ⁸ McKenna, M. C., *Amer. Mus. Novit.*, **2158**, 1 (1963).
- ⁹ van Valen, L., *Evolution*, **18**, 484 (1964).
- ¹⁰ Pilbeam, D., *The Ascent of Man* (Collier-Macmillan, New York, 1972).
- ¹¹ Tattersall, I., *Man's Ancestors* (Murray, London, 1970).

Predicting Technology

Technological Planning and Social Futures. By Erich Jantsch. Pp. xiv+256. (Cassell/Associated Business Programmes: London, March 1972.) £4.50.

THIS book is a masterpiece of overstatement. The author suffers a little from the illusion, common amongst enthusiastic proponents of complex management techniques, that the methods advocated are already successfully and widely practised in government and industry, or soon could be. Thus, for example, the book repeatedly refers to the experience of PPBS as though it played an extremely important part in the decision making of the United States government. No one would ever guess from this set of essays that the general experience of PPBS, however worthy the concept may be, has been one of disillusion, or that it has been largely discarded and down-graded. Even more important, it would apparently never occur to the author that there is more to be learnt about US policy making from the Watergate hearings than from PPBS or PERT.

Similarly, in the case of industry

there is a gross overestimation of the role of "technological forecasting." All the empirical evidence shows that its actual influence on decision making both in America and Europe today is very slight. Few firms employ "technological forecasters", and those which do, take their accountants' advice far more seriously.

Only when he reaches the level of world decision making does a greater degree of realism creep in, although even here Jantsch plays with the notion of the "world corporations" filling the vacuum of rational global policy making. By and large, however, he recognizes that nation states and international corporations do not at present adopt a standpoint of global responsibility, but one of self-interest. This leads him to a position of extreme pessimism, as he accepts uncritically the naive models of Forrester on resource depletion, population and pollution. Chapter 14 is a reprint of his review of Forrester's book. Since he irritatingly equates "rationality" with the adoption of "system dynamics" by policy makers, it is hardly surprising that this chapter strikes a "Gottterdammerung" note markedly contrasting with the earlier chapters.

The style is somewhat ponderous and opaque. It compares in this respect rather unfavourably with the lucidity, scholarship and common sense of Vickers' discussion of similar problems, or Bright's clear, practical explanations or Gabor's lively normative approach to the choice of technology and future social change. Impatience with the unsatisfactory progress and narrowness of all the social sciences is quite understandable and legitimate, but Jantsch is too often inclined to substitute the box-diagram or the organisation chart. Not surprisingly, he has a considerable affinity with Forrester, the leading exponent of the universal application of system dynamics to social problems of all kinds. He shares with Forrester, too, an exaggerated respect for the capacity of American corporations to find solutions for social problems, whether at home or abroad.

All of which is a great pity, because underneath the jargon there is a nugget of gold. Whilst Jantsch suffers in full measure from some of the typical faults of the systems engineer in discussing social systems, his book also has the great virtues of this approach. He cuts through the trees and sees the wood as well. Many of his observations about policy making, new institutions and the complementary relationship of extrapolative and normative forecasting are extremely important.

Interdisciplinary research and functionally-based decision making are both highly desirable in many modern institutions. Although he exaggerates their

contemporary influence, some of the methods and reforms which he advocates are urgently needed. If he could summarize the main theme of these essays, and concentrate on the essential message, it would be a shorter book, but a much better one. C. FREEMAN

Protective Mixture

Radiation Protection: A Guide for Scientists and Physicians. By J. Shapiro. Pp. xx+339. (Harvard University: Cambridge, Massachusetts; Oxford University; London, 1972.) £7.50 boards; £3.50 paper.

THE prospective author of a textbook or manual on radiation protection is required to provide a proper mixture of various disciplines and practices—physics, chemistry, biology, medicine, instrumentation, public relations, legislation and so on. To be truly comprehensive the mixture must be absolutely right but the author has a certain freedom in selecting the extent and the academic level of his compilation.

Dr Shapiro has aimed at a wide range of readers, including "physicians, research scientists, engineers and technicians". Part 1 is a historical prologue ranging from the primordial through the discovery of radiation to a statement of the need for radiation protection and the extraordinary measures—technical and legislative—that are necessary. Part 2 is offered as the core of the manual. It deals with the energy imparted by ionizing particles; with beta, gamma and other radiations; dose from external and internal sources and the significance relative to radiation standards, biological effects and natural background; and the calculation of radiation hazard. In the remaining parts (3–6) the following topics are covered successively: dose calculations; radiation measurements; protocol for users of radionuclides (necessary but hardly exciting); and considerations of public health (this provides valuable perspectives).

The coverage is complete, the style simple, the order logical and the whole easy to read. It is possible to criticize the disproportion in the treatment (under "Radiation Measurements") of G-M and scintillation counters (thirty pages) and that of ionization chambers (three pages). To test the academic level of this book I tried it on a young pre-university science student. He found the text intelligible and the calculations quite easy. He would have appreciated more rigorous mathematical treatments of some of the cases, inserted perhaps as appendices. This is a good manual, however, suitable mainly for the technician but with something in it for specialists from the other disciplines.

D. H. PEIRSON

Immunochemistry

Advanced Immunochemistry. By Eugene D. Day. Pp. xvi+447. (Williams and Wilkins: Baltimore, Maryland, 1972.) \$19.

As suggested by the title, some prior knowledge of immunology is required to appreciate this book fully. The first half of the book deals with the structure of antibodies under four headings—"Light Chains of Immunoglobulins", "Heavy Chains of Immunoglobulins", "Sizes and Shapes of the Immunoglobulins" and "The Antibody Binding Site". In each chapter the past work (particularly from the 1950s-1960s) is introduced and leads to our present state of knowledge (and problems). The second half of the book deals with the reactions of antibodies. Besides the expected applications of law of mass action to reactions of antibodies with haptens and multivalent antigens, there is a very well written and concise chapter on the active centres of multivalent antigens.

Obviously a book bearing this title and running for only 401 pages (exclusive of index) will leave out certain aspects of the subject. The author has pre-empted the words of the critic, however, by mentioning most of these in the last chapter. The first half of the book deserves unqualified approval for its concise and logical presentation of our knowledge up to about the beginning of 1971. It will be very welcome for final degree students specializing in immunology, for people who want their immunological knowledge brought up to date, and of course for PhD students for its review of the development of the various topics. The second half is not so impressive simply because most of the information is available in other books and the information it contains has been around for a longer period of time. But apart from a spelling mistake on page 391, it is a useful review of the subject.

C. A. KING

Sequencing RNA

Determination of Sequences in RNA. By G. G. Brownlee. (Laboratory Techniques in Biochemistry and Molecular Biology, Volume 3, Part I.) Pp. 265. Edited by T. S. Work and E. Work. (North-Holland: Amsterdam, 1972.) £13.30.

Nucleic Acid Sequence Analysis. By Stanley Mandel. Pp. 282. (Columbia University: New York and London, February 1973.) £7.

In recent years great progress has been made in the field of nucleic acid sequencing. Besides the two classical techniques which opened the way to such studies, DEAE chromatography and the fingerprinting method, there are now many modifications in use for

investigating large RNA molecules. New approaches have also been introduced, based on copying *in vitro* RNA or DNA and combining the former analytical techniques with nearest neighbour determination and with kinetic analysis of synchronously synthesized fragments of RNA. The number of RNAs with at least partially known sequences is ever increasing and promising attempts have been made also to apply sequencing techniques to some DNA molecules. A comprehensive review in this field has indeed been needed for a long time and can be expected to be very helpful both to scientists familiar with some aspects of these problems and to those intending to start now nucleotide sequence analysis. It is therefore very welcome that two monographs on RNA sequencing have been published at approximately the same time.

Brownlee's book deals mainly with the two classical methods, Holley's column chromatography and Sanger's fingerprinting technique, and mentions briefly some new approaches involving *in vitro* labelling of RNAs. After a short introduction, he describes in detail the enzymic digestion and chromatographic fractionation techniques used in studying the structure of non-radioactive RNAs and the analytical methods for establishing the structure of the digestion products. Chapters 3-6 give a very comprehensive description of the fingerprinting technique, including technical details, alternative methods, diagrams showing the electrophoretic mobilities of different oligonucleotides and their degradation products. Chapter 5 is perhaps a too lengthy presentation of the sequencing of 5S RNA but it discusses also many modifications of the original technique and gives a useful illustration of the practical application of different analytical methods. Chapters 7 and 8 deal with some special problems, like minor bases and terminal sequences, while chapter 9 summarizes briefly some more recent methods involving *in vitro* labelling of RNAs.

Throughout the work the practical side of the problems is emphasized, and results are discussed only as far as they facilitate the understanding of the methods used. In addition, there is a very useful appendix, giving further details about the exact conditions for labelling, isolating, fractionating, digesting RNAs, mentioning even the suppliers of equipment, enzyme preparations and other special chemicals used. The book is certainly a very useful manual, enabling the reader to set up a suitably equipped laboratory and start sequence studies on RNA.

The intentions of Mandel's book seem different. He reviews a much wider field of sequence work, covering

at least the principles of almost all techniques ever used for partial or total sequence determination. He even includes early approaches which had rather limited use and a chapter on physical methods which are at present not suited for sequence studies but might be further developed and possibly used in the future for such purposes. The first nine chapters describe the principle and occasionally also some practical details of all the sequencing methods applied to RNA; chapters 10 and 11 give practical description of several techniques used in RNA research, from isolation techniques of RNA to analysis of some small degradation products. The choice of techniques presented in this latter part is not very fortunate: some simple, widely known procedures are described in detail while some specialized techniques are mentioned rather briefly. The last chapter summarizes the results of RNA sequencing: the structures of a great number of RNAs, as known today, are shown.

As the first review covering this whole field the book is certainly of considerable interest. It is not very well suited, however, to be used as a manual or as a guide to find the best technique for a particular sequencing problem. This is partly because the practical information is sometimes insufficient, partly because different methods are not always critically compared and evaluated with respect to their application to different problems.

M. SZEKELY

Inorganic Systems

The Constitution of Inorganic Compounds: Atomic Quantum Mechanics—Metals and Intermetallic Compounds. By John L. T. Waugh. Pp. xv+797. (Wiley: New York and London, August 1972.) £12.50.

THIS is an immense book, but it is only the first of two volumes devoted to inorganic molecules and solids. This first volume concentrates on the fundamentals of quantum theory, so that by page 797 the reader has just been prepared for a subsequent study of inorganic systems. It is a pity that both volumes could not have been published simultaneously, for one could then see more clearly the rationale of the choices of topic in volume 1.

In brief the author presupposes no prior knowledge of quantum theory. He introduces it from scratch, developing the general principles of the wave equation, operators, mean values, particle-in-a-box, hydrogen atom, and so on, following rather standard analysis. There follow chapters on variation and perturbation methods, spin, and atomic structure. By now we are at page 379 and halfway through the book. The second half is devoted to the metallic

state, band structure, Brillouin zones and a little about intermetallic compounds.

This is a formidable amount of material, especially when one sees what the author means when he writes in the preface that he wants to discuss inorganic systems "without simply stating results". He has certainly achieved this aim: there are three pages showing line by line how to pass from Cartesian to spherical polar coordinates in the expansion of ∇^2 ; and there are four pages in small type working in detail through the Kronig-Penney model of a lattice.

It is impossible in a book of this kind to keep a uniform mathematical level. Thus the calculus of variations, which is hard, comes on page 103, just ten pages before the use of an Argand diagram, which is easy. There does not seem to be much that is wrong, though someone new to the Dirac δ -function might be surprised to see it defined (page 131) by $f(x) = \int f(x) \delta(x-x) dx$. Almost inevitably there is a certain inelegance when so much ground has to be covered (for example, the proof of the Uncertainty Principle on page 158).

On the good side it must be said that most of the mathematics really is clear, and the diagrams are excellent, so that if a student were to take up this book and work steadily through it, without any doubt he would be able to learn a lot. It would be an excellent book, not so much for class use, but as a reference volume to which a student could be referred if he found his class lectures unpalatable. C. A. COULSON

Ornithological Statistics

The Visible Migration of Birds at Ottenby, Sweden. Edited by Carl Edellstam. Drawings by Harald Wiberg. Pp. 360. (Swedish Ornithological Association: Stockholm, 1972.) 95 Sw. cr.

THIS is an extraordinary book in several senses of the word. Teams at many bird observatories have counted the birds daily migrating past. In the few cases where analyses have been published it is usually stated that the original data have been deposited at some institute. The Ottenby team decided that this was not good enough and have deposited the original data in the reader's lap. Interpretation and commentary occupy less than a fifth of the book—a tenth, really, since each page is half English, half Swedish.

The data concern ten years, 1947 to 1956, June to October, and 1,509 observation days. The yearly totals for sixty species are presented in the form of histograms elegantly spaced over ten pages. These are then re-sorted into a further set of 10-day period histograms. Then the daily totals for each species

for each year are set out, again as histograms, over 180 pages. The monotony of page (36×22.5 cm) after page of black bars and white space is offset by an elegant line drawing of each species.

Next the hourly variations within each 10-day period are presented in the form of two seven-step ladders, on the rungs of which the histograms are mounted. The extravagance of the layout is even more apparent here, for in half the 70 species given a page apiece, the left or right hand ladder is completely empty.

The production is impeccable, the paper quality high, the draughtsmanship superb, the amount of work appalling. Yet what does it achieve? Certainly, as pointed out in the commentaries, impressions can be gained by visual inspection, but to test them recourse to the original numerical data is still needed. These could be extracted by measurement (the histograms are meticulously accurate) but it would have been simpler (and not much duller) to have the numbers themselves, and to use histograms to illustrate particular points that statistical analysis had shown to be valid.

There is little doubt that this book will remain unique. A wide sale cannot be forecast. G. V. T. MATTHEWS

Geometrical Optics

The Optics of Rays, Wavefronts and Caustics. By O. N. Stavroudis. Pp. xvi+313. (Academic: New York and London, October 1972.) \$18.

THIS interesting and unusual book on geometrical optics begins (after an historical introduction) by obtaining the concept of a ray path in a medium of continuously varying refractive index via the calculus of variations, and continues with an account of the differential geometry of curvature and torsion of generalized space curves. Then the Hilbert integral is defined and used to deduce the mathematical concepts of the wavefront as a transversal of a two-parameter family of rays and the caustic as the envelope of such a family. Snell's law is then derived for a medium of sectionally-continuous refractive index.

Contact with the bread-and-butter of geometrical optics is made in a discussion of ray tracing, but the treatment is at an esoteric level with no real mention of how to do ray tracing in cases of practical interest. It is probably fair to assume, however, that no neophyte in need of such advice would buy the book anyway.

Chapter seven establishes the general condition for the existence of a wavefront to a set of rays and Malus's theorem is formally proved. The chapter on "Generalized Raytracing" concerns the propagation of an element of wavefront through a system and represents, in effect, a generalized paraxial

Just released . . .

LIQUID CRYSTAL DEVICES

Thomos Kallard, editor

CONTENTS: Four state-of-art papers by J. A. Castellano; J. M. Washick; F. C. Kahn, D. Maydan, G. N. Taylor; C. R. Stein. The bibliography contains 1100 entries from world-wide sources. Over 150 U. S. and foreign patents described, 450 illustrations, 392 pages. Index. \$15.00 post-paid on prepaid orders (ISBN 0-87739-007-X)

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ray trace in which the optical axis is replaced by a skew finite ray.

The discussion on classical aberration theory includes Hamilton's characteristic function, Hertzberger's diappoint theory, and an incomplete account of the Seidel aberrations. This is preceded by a partially successful attempt to produce the reverse of the well-known Luneburg derivation of the fundamental properties of geometrical optics from Maxwell's equations.

A radically different mechanism of image analysis is then studied in the form of Hertzberger's fundamental optical invariant and its scalar form the lens equation. This is a system of first-order partial differential equations involving ray coordinates and directions at the object and image planes. The book closes with a closely-reasoned but slightly tongue-in-cheek application of algebraic group theory to lenses—heady stuff for lens designers.

In summary, there is little in the book to cause the designer to rush to the coding sheet to rewrite his programs—it is not a book of that level. But for those of us who enjoy an occasional dip into the rigours of Luneburg there is much here to interest and stimulate, and the author succeeds in guiding us through the welter of mathematics with an entertaining and readable style. JOHN MACDONALD

CORRESPONDENCE

Misidentification or Misunderstanding?

SIR,—The article 'Misidentified Dyke' (*Nature*, **244**, 485; 1973) by your geomagnetism correspondent contains the following inaccuracies. (1) The trend of the Wackerfield Dyke is ENE not WNW (Fig. 1). It is important because dyke trends in Northern England have long been recognised as reliable indications of age, WNW for Tertiary and ENE for late Carboniferous (called Carboniferous-Permian before the advent of radiometric age determination). (2) It is not therefore "fairly obvious from field relations that it forms part of the Cleveland-Armathwaite Dyke"; their outcrops are near but their trends are different. The original statement by Tarling *et al.*¹ that "its field relations suggest that it could be part of

the Cleveland-Armathwaite Dyke" is only slightly less misleading. (3) Tarling *et al.* do not claim that the dyke has "always been regarded as part of the Tertiary activity", nor do I know of a publication which does. On the contrary it is unequivocally grouped with the dykes associated with the Upper Carboniferous Whin Sill and equally unequivocally differentiated from the Tertiary Cleveland Dyke in the standard petrographic studies of Holmes and Smith² and Holmes and Harwood³—both quoted by Tarling *et al.* It is also clearly shown as of 'Carboniferous-Permian' age on the Geological Survey one-inch map (Sheet 32: Barnard Castle) published in 1969.

The work of Tarling *et al.* thus con-

firms "long cherished beliefs" rather than requires that they should be discarded. In case it is overlooked, it may be as well to emphasise the real contribution made by Tarling *et al.*—namely the relevance of palaeomagnetic studies in establishing the age of emplacement and subsequent history of intrusions like the Wackerfield Dyke.

Yours faithfully,

D. A. C. MILLS

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Ring Road, Halton,
Leeds LS15 8TQ*

¹ Tarling, D. H., and Mitchell, J. G., *Earth planet. Sci. Lett.*, **18**, 427 (1973).

² Holmes, A., and Smith, S., *Geol. Mag.*, **58**, 440 (1921).

³ Holmes, A., and Harwood, H. F., *Mineralog. Mag.*, **21**, 493 (1928).

Our Geomagnetism Correspondent writes:

Dr Mills is quite right; I have misunderstood. I measured the trend of the Wackerfield Dyke from the Tarling *et al.* map which, because of its small scale, led to the ambiguity now resolved by Dr Mills' larger scale version. Once this point is cleared up, and accepting the points made by Dr Mills in (3) above, it becomes difficult to understand why Tarling *et al.* should say that "its (the Wackerfield Dyke's) field relationships suggest it could be related to the Eocene Mull Dyke Swarm"—a misleading statement which, compounded by my error in the trend, I am guilty of accepting rather too uncritically.

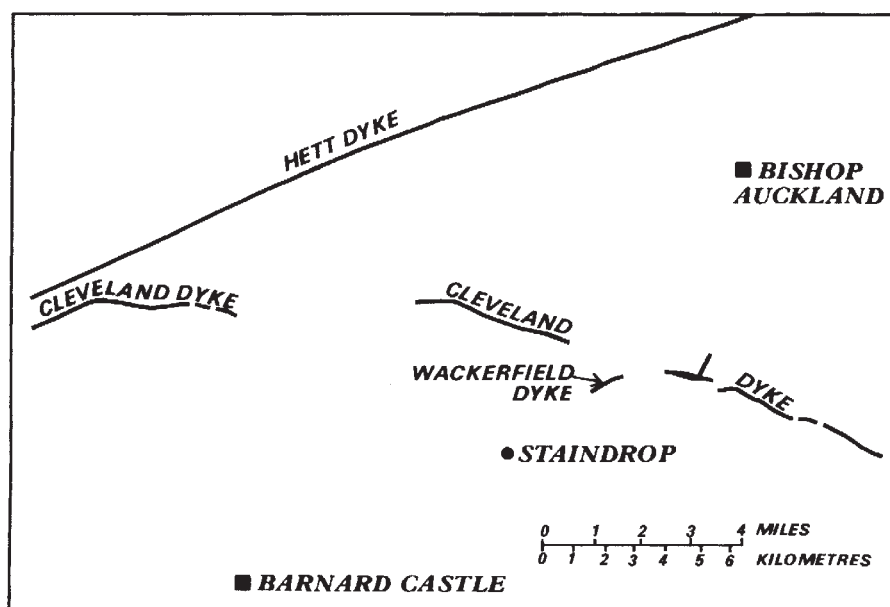


Fig. 1 Map of southern part of Co. Durham showing outcrops of the Hett, Wackerfield and Cleveland Dykes.

Obituary

Professor Jean Hanson

Professor Emmeline Jean Hanson, FRS, Director of the Medical Research Council's Muscle Biophysics Unit at King's College London, died on August 10 of an acute meningococcal infection, at the age of 53.

An only child, she was born in Derbyshire, and was educated at the High School for Girls, Burton-on-Trent, and

at Bedford College in the University of London. After taking her BSc in Zoology, she was engaged in cancer research for two years at the Strangeways Research Laboratory, Cambridge, producing two papers on the histogenesis and differentiation of the mammalian epidermis. She then returned to Bedford College in 1944 with a teaching appointment in the Zoology Department, making a number of histological

and other investigations on several aspects of structure and function in annelids.

The decisive step in her scientific career was her appointment in 1948 to the Biophysics Research Unit, established by the Medical Research Council in the previous year under Sir John Randall, then Wheatstone Professor of Physics at King's College London. She and Randall were put in touch by

Munro Fox, the Head of her Department at Bedford College, and the appointment was as significant for the unit as it was for her. Her wide knowledge of biology was an important factor in a unit where other members had almost all been trained in the physical sciences, and her personal qualities—her energy, her integrity and her helpfulness to others—contributed greatly to the successful development of the unit. The investigations on muscle, which later became one of the chief interests of the unit, were centred on her from the beginning. For the first few years she was the only member of the unit working on muscle, but under her inspiration this side of the unit's activity expanded so that, when the unit was divided after Randall's retirement in 1970, one part became the Muscle Biophysics Unit, and she was appointed as its Director.

Although she held a full-time research appointment, she devoted a large part of her energy to undergraduate teaching at King's College. Not only did she develop courses in Biophysics, both within King's and as part of the inter-collegiate teaching of London University, but she played a key part in the development of the School of Biological Sciences at King's. She often acted as an external examiner, both for undergraduate examinations and for doctoral theses, and made a reputation for sympathetic and conscientious work in this capacity. In 1966, the University of London conferred on her the title of Professor of Biology.

From the time when she first joined Randall's Biophysics Research Unit, almost all of her research was concerned with the structural basis of muscular contraction, and it is for this that she will chiefly be remembered. Her first

attempts at elucidating the changes in the striation pattern during contraction, published in 1952, showed the formation of contraction bands but threw no light on the earlier stages of shortening. Her method, however—observing separated myofibrils under a phase-contrast microscope—was used again by her with great success in a period of highly fruitful collaboration with Dr H. E. Huxley. This began in 1953–54, when both of them spent the year in the laboratory of Professor F. O. Schmitt at the Massachusetts Institute of Technology, she as a Fellow of the Rockefeller Foundation. They first established the localisation of myosin and actin in the striation pattern (also done independently at the same time by W. Hasselbach in H. H. Weber's laboratory) and soon afterwards showed the constancy of the distances in the striation pattern corresponding to the lengths of the thick and thin filaments. This was paralleled in part by A. F. Huxley and R. Niedergerke's measurements on living fibres. Together with H. E. Huxley's evidence from electron microscopy and X-ray diffraction, these results formed the chief basis of the sliding filament theory, now universally accepted.

Her later work, much of it in collaboration with Professor J. Lowy, was almost all carried out with the electron microscope. Most of it was related to two problems: the structure of muscles of molluscs and other invertebrates, and the structure of the thin filaments. Hanson and Lowy were the first to obtain isolated thin filaments, and these showed clearly the helical arrangement of the actin monomers. Later work, on which she was still engaged at the time of her death, was concerned with the position of other components of the thin fila-

ments, tropomyosin and troponin.

Aside from her own investigations, she played a very great part by inspiring the muscle team that she gradually built up. Her name appeared on papers only when she had actually taken a major part in the experimental work, but all the other work from the unit, whether electron microscopy, X-ray diffraction or protein chemistry, owed a tremendous amount to her interest, encouragement and criticism, and to her wide knowledge both of the muscle field and of biology in general. Under the influence of her personality, the unit became an exceptionally happy place, where there was excellent cooperation between the members, and where visitors were made welcome.

She was elected to the Royal Society in 1967. She was outstandingly friendly and helpful towards everyone she had to do with, and will be deeply missed by a wide circle of scientific colleagues and other friends. She was unmarried.

Announcements

University News

Professor C. Michael has been appointed to the chair of theoretical physics, **University of Liverpool**.

Dr J. T. Owen has been appointed to the chair of anatomy, **University of Newcastle upon Tyne**. **Professor A. Mitchell** has been appointed to the William Cochrane Chair of Metallurgy and Engineering Materials, **University of Newcastle upon Tyne**.

Professor W. Williams has been appointed Professor of Agricultural Botany, **University of Reading**.

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